

# Ant interceptions reveal roles of transport and commodity in identifying biosecurity risk pathways into Australia

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## Abstract

We obtained 14,140 interception records of ants arriving in Australia between 1986 and 2010 to examine taxonomic and biogeographic patterns of invasion. We also evaluated how trade and transport data influenced interception rates, the identity of species being transported, the commerce most associated with the transport of ants, and which countries are the primary sources for ants arriving in Australia. The majority of ant interceptions, accounting for 48% of interceptions, were from Asia and Oceania. The top commodities associated with ant interceptions were: (1) Live trees, plants, cut flowers; (2) Wood and wood products; (3) Edible vegetables; and (4) Edible fruit and nuts. The best fitting model for predicting ant interceptions included volumes for these four commodities, as well as total trade value, transport volume, and geographic distance (with increased distance decreasing predicted ant interceptions). Intercepted ants identified to species consisted of a combination of species native to Australia, introduced species already established in Australia, and species not yet known to be established. 82% of interceptions identified to species level were of species already known to be established in Australia with *Paratrechina longicornis* having the most records. These data provide key biogeographic insight into the overlooked transport stage of the invasion process. Given the difficult nature of eradication, once an ant species is firmly established, focusing on early detection and quarantine is key for reducing the establishment of new invasions.

**Keywords**

*Anoplolepis gracilipes*, biological invasions, interception records, introduced ants, *Iridomyrmex purpureus*, *Linepithema humile*, *Monomorium pharaonis*, *Pheidole megacephala*, ports of entry, *Tapinoma melanocephalum*

**Introduction**

Biological invasions are a global economic and ecological threat (Vitousek et al. 1996; Mack et al. 2000; Pimentel et al. 2005; Bellard et al. 2016). Insular environments are particularly vulnerable to invasion; due to their high levels of endemism the loss of beta diversity results in global biotic homogenization (Case 1996; McKinney and Lockwood 1999; Kier et al. 2009; Ricotta et al. 2014; Tershy et al. 2015; Moser et al. 2018). Given that invasive species are difficult to eradicate once established, prevention is key to minimizing their consequences (Simberloff et al. 2013; Hoffmann et al. 2016). Risk assessment strategies for invasive species therefore often prioritize identifying sources and pathways of invasion (Anderson et al. 2004; Hulme et al. 2008). Identifying vectors, commerce, and regions that are sources of introduced species will provide additional benefits. For example, examining records of species intercepted during transport can provide information on how introduction effort (e.g., propagule supply) influences establishment success (Cassey et al. 2004; Lockwood et al. 2005), and identify biogeographic patterns of invasion providing key insight into mechanisms of their success (Cassey et al. 2005; Hulme et al. 2008; Brawley et al. 2009; Ricotta et al. 2014; Moser et al. 2018).

Ants are among the most widespread and costly invasive species (Holway et al. 2002, Rabitsch 2011). Over 150 ant species have become established outside their native range, and island ecosystems appear particularly susceptible (McGlynn 1999; Suarez et al. 2010). For example, many islands that historically maintained unique, depauperate or no native ant diversity, now harbor communities dominated by introduced species (Morrison 1996; O'Dowd et al. 2003; Krushelnycky et al. 2005; Smith and Fisher 2009; Ward 2009; Cerdá et al. 2012; Sarnat and Economo 2012; Hoffmann et al. 2017). Despite over a century of research on the consequences of ant invasions, we know relatively little about which taxa are transported by human commerce or the biogeographic patterns of ant introductions. However, recent efforts examining ant interception records have shed light on these issues (Lester 2005; Ward et al. 2006; Suarez et al. 2010; Miravete et al. 2013; Bertelsmeier et al. 2018). For example, ants intercepted in quarantine in the United States suggest introductions do not follow a biogeographic pattern typical of other introduced insects in North America (Suarez et al. 2005; Bertelsmeier et al. 2018); while most insect invasions to the Nearctic region historically originated from the Palearctic (Sailer 1978) and most ants transported and introduced to the U.S. originate from the Neotropics (McGlynn 1999; Suarez et al. 2005). By contrast, most ant interceptions in New Zealand originate from the Pacific despite the species transported not being native to that region (Ward et al. 2006; Bertelsmeier et al. 2018). Taken together, these studies suggest that patterns of ant introduction, and subsequently risk assessment, may be regionally specific.

Australia is the world's largest continental island, and has biosecurity standards considered to be among the most stringent in the world (Meyerson and Reaser 2002; Wilson and Weber 2002). Over 100 ant species were intercepted at Australian ports from 1986–2002, with accelerated rates in the last five years accounting for 90% of all interceptions (Commonwealth of Australia 2006). Consequently, Australia provides an exceptional case study through which we can better understand patterns of ant invasions, providing valuable data to assist biosecurity managers and policy makers. We used a dataset of over 10,000 interception records of ants arriving in Australia via human commerce to examine biogeographic patterns of invasion. We compared these records to trade and transport data over the same time frame to examine how these factors influence interception rates and the identity of species being transported. We also determined what commerce is most associated with the transport of ants, and what countries are the primary sources for ants arriving in Australia. These data provide key biogeographic insight into the overlooked transport stage of the invasion process.

## Methods

### Interception data

Interception records were sourced from the Australian Government Department of Agriculture and Water Resources Pest & Disease Information database (PDI) (1986–2003), and the Incidents database (2003–2010), which replaced PDI. Interception data included information on the following: date, location (source country and arrival state in Australia), transport vector (air/sea), associated traded commodities that the ants were intercepted with, identification to the lowest taxonomic level possible (e.g. species/genera/subfamily), animal condition (alive/dead), life stage (egg/larva/pupa/adult), and caste (worker, alate or dealate queen).

For each record, which was identified to species or genera, the record was placed into one of four discrete categories describing its status in Australia:

***endemic*** – if range of species or genus is only known to occur within Australia;

***native*** – for species/genera whose native range includes Australia;

***introduced*** – for species/genera established in Australia but whose native range is outside of Australia;

***not established*** – for species/genera whose native range is outside of Australia and are not known to have yet established populations in Australia.

This information was determined using databases and literature of species and genera known to occur in Australia (e.g. Anderson 1991, 2000; Shattuck 1999; <https://www.antweb.org>, <https://www.antwiki.org>).

For most records, ants were only identified to genus. Exceptions included the most commonly intercepted introduced ant species for which diagnostic guides are available to staff (e.g., black crazy ant [*Paratrechina longicornis*], yellow crazy ant

[*Anoplolepis gracilipes*], coastal brown ants/big headed ant [*Pheidole megacephala*], Singapore ant [*Trichomyrmex destructor*], Pharaoh's ant [*Monomorium pharaonis*], and Argentine ant [*Linepithema humile*]). Even for these species, expertise may be port specific. For example, the Argentine ant is well established and common around port areas in Melbourne while more tropical species like the black crazy ant are more commonly seen in Brisbane. Consequently, intercepted ants may be more likely to be identified to species in areas where they already occur. Common or easily recognized native species were also often identified to species or species group (e.g., *Iridomyrmex purpureus*). Finally, reproductive castes (e.g., winged alates and dealate queens) were often not identified beyond family level (A. Broadley Pers. Comm.).

### Trade and transport data

We extracted the import value (US\$) of merchandise trade (AG2 classification code) with Australia's trading partner countries from the United Nations Commodity Trade Statistics Database (UN Comtrade) for the years 1988–2015. Commodity descriptions associated with ant interceptions were standardized to match these AG2 classification codes. GDP per capita (current US\$) data were obtained from the World Bank national accounts data, and OECD National Accounts data files available from 1960–2013 (<http://data.worldbank.org/indicator/NY.GDP.PCAP.CD>, accessed 29/09/2014). We used trade data from 2010 for all calculations. For physical international transport into Australia we obtained: (1) shipping data from the Australian Government Department of Agriculture and Water Resources; and (2) flight information data from OAG Aviation (<http://www.oag.com>), over the time period 1999–2012. Flight and shipping data were combined into an integrated physical transport metric by taking a weighted sum,  $I = w_s \times \text{number of ships} + w_p \times \text{number of passenger flights} + w_c \times \text{number of cargo flights}$  (*sensu* Cope et al. (2016)), with weights ( $w_s, w_p, w_c$ ) chosen so that the three transport pathways contributed in proportion to the total number of ant interceptions associated with that pathway.

### Data analysis

We analyzed these data for summary statistics and general trends relating to ant interceptions into Australia over time. We also identified pathways and commodities associated with high levels of ant interceptions. We used Poisson regression to model the number of ant interception records, testing predictors including: integrated physical transport into Australia (flights and ships), and trade value into Australia (both total trade value, and trade associated with key commodities), to highlight high risk pathways and commodities. Geographic distance to Australia, and GDP per capita of source countries were also tested as possible predictors. Finally, we calculated the Shannon diversity index per year (using the 'vegan' package in R; Okasanen et al. 2018) for ants from the introduced category that were identified to species, and the diversity per region of detected genera.

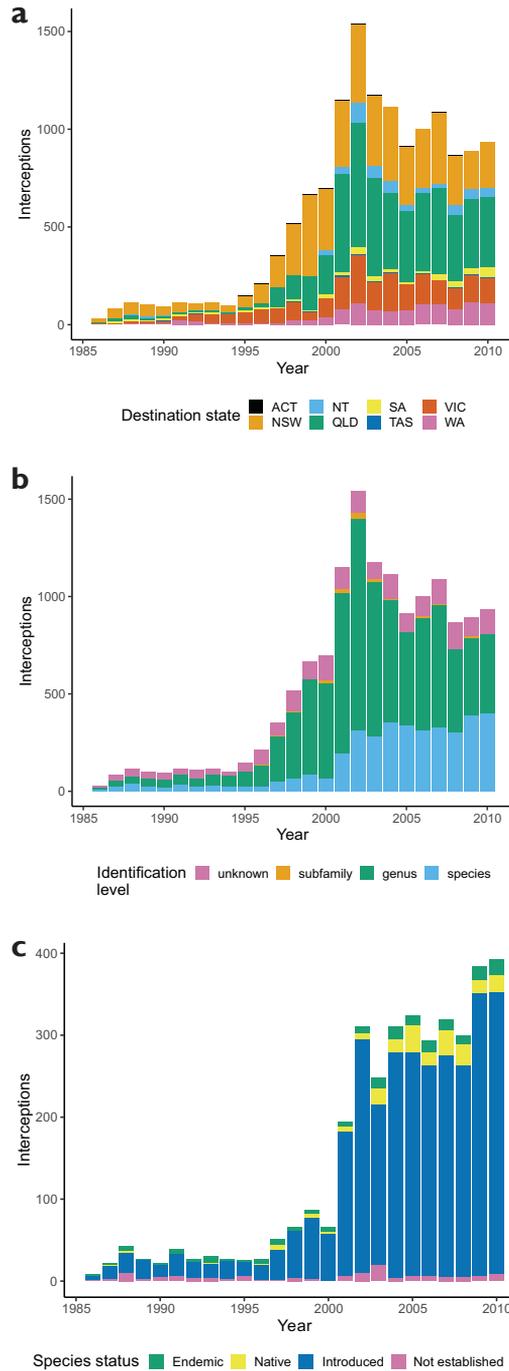
Model selection was performed using Akaike Information Criterion (AIC), with the model producing the minimal AIC being chosen (Akaike 1974). We report AIC and Akaike weights for competing models, and regression parameters (both raw, and with continuous parameters standardized to have mean zero and standard deviation 1) for the final model. Models with interaction terms were excluded to allow clear interpretation of parameters and avoid overfitting. A binary indicator for the presence of any physical transport from the source country to Australia was used as an additional predictor, as many countries had no recorded (direct) transport. All analysis was performed in R 3.5.1 (R Core Team 2018).

## Results

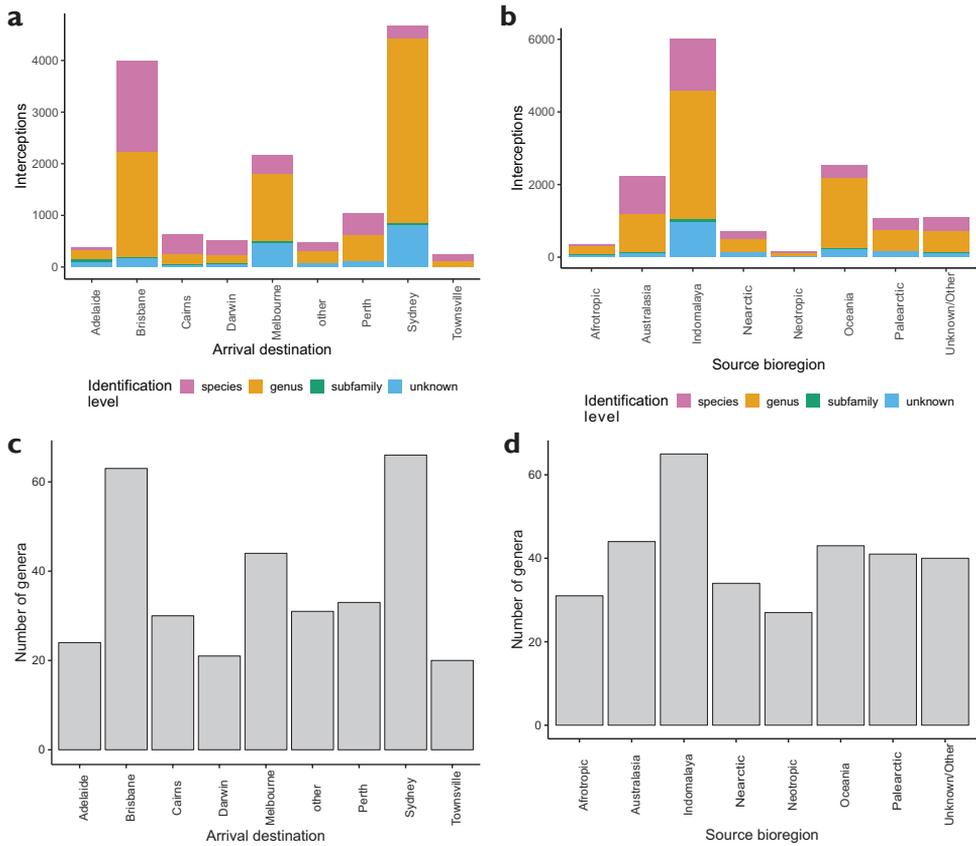
### Interception and identification summary

We obtained 14,140 interception records between 1986 and 2010. The number of recorded ant interceptions was relatively low from 1986–1997 (with a mean of 112 interceptions per year across this time period) before increasing to a peak of 1541 interceptions in 2002. The number of interceptions then levelled off to an average of 998 interceptions per year from 2002–2010 (Figure 1a). Most recorded ant interceptions included adult ants (92.5% of records), although substantial proportions of interceptions included eggs (15.5%), or larva / pupa (19.5%) (many records included different life stages). Most interceptions included live ants (90%). Castes were unknown for 86.6% of interceptions, but those interceptions for which they were recorded included a variety of forms including workers, and alate and dealate queens. Overall, 90 different genera and 104 species or species groups were identified from these samples (Suppl. material 1: Table S1). More than half (59%) of the interceptions were identified to genus, 27% to species, and 14% were unidentified (Figure 1b). There was relatively little variation through time in the status of intercepted species; most intercepted species were in the “introduced” category defined as being non-native but already established in Australia (Figure 1c).

There was considerable variation in interceptions among ports of entry and biogeographic region of origin (Figure 2). The smallest proportion of ant interception records identified to species level was in Sydney (5.2%), followed by Adelaide (15.0%) and Melbourne (16.3%). Records were most frequently identified to species level in Cairns (59.9%), and Darwin (53.5%). Brisbane had 44.1% of records identified to species level, despite having the second largest number of total interceptions, behind Sydney (Figure 2). The number of ants intercepted from each country was correlated with the number of genera that made up those interceptions ( $n = 209$  countries, correlation coefficient 0.80; Figure 3), suggesting that generic diversity was primarily due to increased interceptions rather than some source or pathway-specific factor. The top 20 sources for ant interceptions accounted for approximately 48% of interceptions; all originated in Asia or Oceania (specifically Fiji), and the top 7 occurred in either Brisbane or Sydney (Table 1).



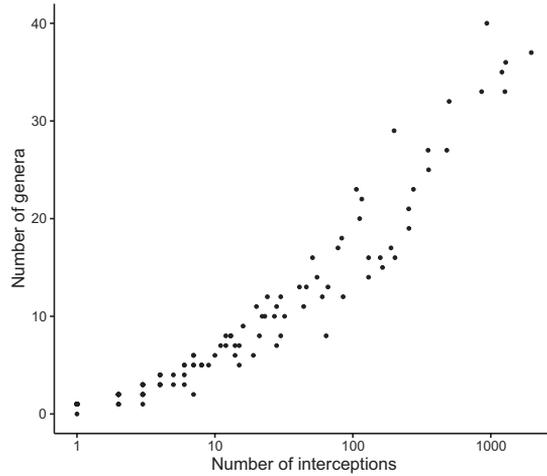
**Figure 1.** Ant interceptions into Australia by year and **(a)** State in which the interception occurred (ACT = Australian Capital Territory, NT = Northern Territory, SA = South Australia, VIC = Victoria, NSW = New South Wales, QLD = Queensland, TAS = Tasmania, WA = Western Australia), **(b)** taxonomic level the interception was identified to, and **(c)** species status for records that were identified to species level.



**Figure 2.** Taxonomic patterns of Australian ant interception data. Taxonomic level identified for interceptions separated by (a) city of arrival (e.g., port of entry) and (b) source biogeographic region. The number of genera identified in interceptions by (c) city of arrival and (d) source bioregion.

## Trade and transport data

Over half (57%) of the ant interceptions were associated with air traffic, 40% were from seaborne traffic, and the remaining 3% were listed as ‘other’, including international mail and records with no listed vector. In contrast to the pattern in interceptions, which leveled off between 2005–2010, the amount of air and sea traffic into Australia continued to increase from 2003–2010 (Suppl. material 2: Figure S1). Most interceptions were from Asia (48%) or Oceania (33%) (Table 1; Figure 2). Interceptions from Asia and Oceania were more likely to be associated with air traffic than those from Europe or the Americas (Suppl. material 3: Figure S2). The number of interception events per country-of-export was positively correlated with both total weighted transport (correlation coefficient 0.40, Figure 4a) and total import value of traded commodities into Australia from that country (correlation coefficient 0.36; Figure 4b). The volumes of transport into Australia along each transport pathway (i.e.,

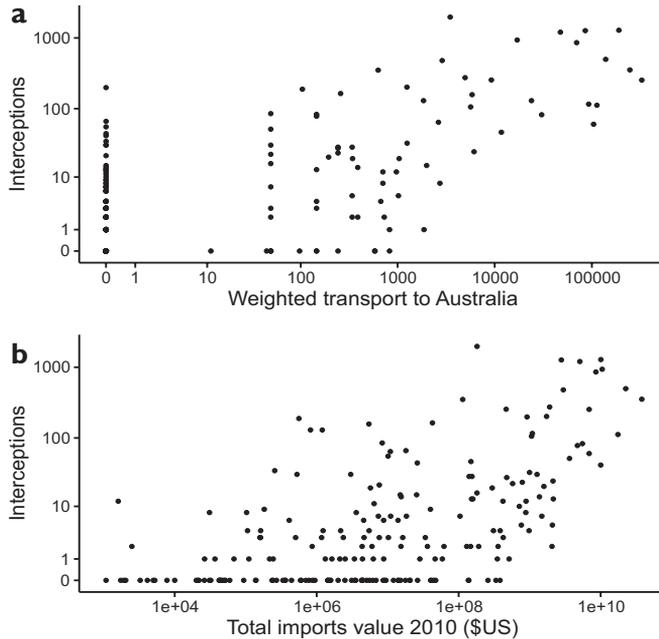


**Figure 3.** The number of genera observed within ant interceptions from a country of origin, correlated with the number of interceptions detected from that country (n = 209, correlation coefficient 0.80).

**Table 1.** Top 20 pathways for commerce on which ants were intercepted in Australia from 1986–2010. From a total of 14140 records, these top eight source countries account for approximately 48% of interceptions.

Source country	Port of entry	Interceptions
Fiji	Sydney	1528
Papua New Guinea	Brisbane	730
Thailand	Sydney	440
Singapore	Brisbane	413
Other	Brisbane	388
Fiji	Brisbane	316
Indonesia	Sydney	272
Singapore	Perth	265
Malaysia	Sydney	250
Papua New Guinea	Cairns	247
Singapore	Sydney	236
Singapore	Melbourne	235
Malaysia	Melbourne	221
Indonesia	Brisbane	201
Indonesia	Melbourne	192
Sri Lanka	Sydney	190
Thailand	Melbourne	189
Indonesia	Cairns	183
Vietnam	Melbourne	183
Malaysia	Brisbane	176

shipping, passenger flights, cargo flights) were also each positively correlated with ant interceptions: the number of ant interceptions per country-of-export had correlation coefficients of 0.33 with the number of ships originating in that country, 0.51 with the number of passenger flights, and 0.35 with the number of cargo flights.



**Figure 4.** Ant interception counts correlated with (a) weighted transport volumes and (b) import value in 2010, by country. Correlation coefficients 0.36 for transport volume and 0.40 for import value.

## Commodities

The top 10 commodities associated with the most interceptions covered 65% of all interceptions (Table 2). These 10 commodities accounted for 4.8% of total imports into Australia by value in 2010, and in general these commodities were not the imports of highest value. An additional 22% of ant interceptions were not associated with any of the listed commodities (e.g., goods unknown); these interceptions were rather associated with the transport mechanism itself, particularly shipping containers, vessels, air baggage, and personal luggage.

The majority of ant interceptions associated with plant and animal products were with products transported by air, except for those associated with timber products, which were mostly transported by sea. The four most common commodities in terms of ant interceptions were: (1) Live trees, plants, cut flowers; (2) Wood and wood products; (3) Edible vegetables; and (4) Edible fruit and nuts (Table 3). In some cases, high trade volumes of these commodities into Australia corresponded to high numbers of associated ant interceptions (e.g., Indonesia was the third largest source of wood products by value, and the highest source of ant interceptions associated with this commodity), but in other cases, countries with the highest trade volumes were not those with the most ant interceptions (e.g., the Netherlands was the greatest source of live trees and cut flowers, but had few associated ant interceptions).

**Table 2.** The top 20 commodity groups for ant interceptions in Australia from 1986 to 2010.

Commodity	Ant interceptions	Proportion of interceptions (%)
Live trees, plants, bulbs, roots, cut flowers, etc.	2599	19.3
Wood, and articles of wood, wood charcoal	1829	13.6
Edible vegetables and certain roots and tubers	1786	13.3
Edible fruit, nuts, peel of citrus, fruit melons	828	6.1
Vegetable plaiting materials, vegetable products	781	5.8
Residues: wastes of food, industry, animal fodder	422	3.1
Meat, fish, and seafood food preparations	333	2.5
Cereals	271	2.0
Vegetable, fruit, nut, etc. food preparations	187	1.4
Vehicles other than railway tramway	177	1.3
Plastics and articles thereof	171	1.2
Boilers, machinery, etc.	140	1.0
Fish, crustaceans, molluscs, aquatic invertebrates, etc.	137	1.0
Miscellaneous edible preparations	122	0.9
Products of animal origin	108	0.8
Coffee, tea, mate and spices	98	0.7
Salt, sulphur, earth, stone, plaster, lime and cement	73	0.5
Dairy products, eggs, honey, edible animal products	71	0.5
Ores, slag and ash	68	0.5
Miscellaneous manufactured articles	60	0.4
Unknown	3159	23.5

The trade value of these common commodities increased over time, but the relative proportions of each commodity remained consistent (Suppl. material 4: Figure S3). In contrast, ant interceptions associated with these commodities varied though time in both magnitude and relative proportion (Figure 5). For example, the proportion of interceptions associated with edible vegetables varied from 1% in 1991 to a peak of 37% in 1999, whereas edible fruit were more consistently between 2–14% of interceptions in every year.

### Statistical model for ant interceptions by country

The best fitting model included each of the top four commodity volumes, along with total trade value (\$US), transport volume (and an indicator variable for non-zero direct transport to Australia), GDP per capita, and geographic distance (Tables 4, 5). Geographic distance had the largest magnitude coefficient after standardization, with an increase in distance resulting in a decrease in predicted ant interceptions (Tables 4, 5). Total trade value, and the values of each commodity, also had large coefficients. However, the coefficient of ‘Edible vegetables’ was negative, likely because the greatest value source countries of this commodity do not correspond to the majority of ant interceptions associated with it (Table 3). GDP per capita had a small positive standardized coefficient. The indicator variable for having non-zero transport to Australia had a relatively large coefficient, whereas transport volume itself had a very small negative coefficient: indicating that direct transport from a country substantially increased the frequency of interceptions from that country (relative to

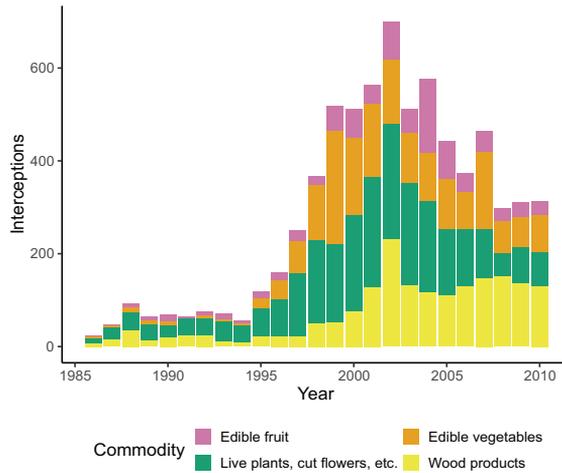
**Table 3.** For the four commodity groups associated with most ant interceptions into Australia, the top ten countries of origin ranked by number of ant interceptions (left column) or the overall value of the imported commodity (right column).

Commodity	Top countries (interceptions)	Number of interceptions	Top countries by trade value	Trade value US\$
Live trees, plants, cut flowers, etc.	Singapore	834	Netherlands	18118004
	Thailand	345	Singapore	10148629
	Malaysia	331	Malaysia	4003579
	Sri Lanka	144	Kenya	3548410
	Indonesia	96	Colombia	2928767
	Fiji	80	India	1829210
	Vietnam	69	China	1757426
	Papua New Guinea	67	New Zealand	1721401
	United States of America	65	Thailand	1568278
	Kenya	53	Chile	1378043
Edible vegetables	Fiji	1407	New Zealand	79814617
	Thailand	90	China	67045088
	Tonga	56	USA	24352741
	Singapore	33	Canada	7452113
	China	22	Peru	6905847
	France	16	Turkey	6766208
	New Zealand	15	Mexico	6207453
	Indonesia	12	India	5854088
	Malaysia	12	Thailand	5682768
	Australia	9	Fiji	5394880
Edible fruit, nuts, etc.	Thailand	198	USA	135207706
	Fiji	96	New Zealand	87758680
	Papua New Guinea	59	Viet Nam	83315454
	Samoa	49	Areas not elsewhere specified	62127512
	United States of America	47	Turkey	44986111
	Tonga	42	China	44272718
	New Zealand	40	Chile	20272657
	Vietnam	28	Thailand	11017653
	American Samoa	26	Philippines	10908714
	Indonesia	25	Italy	9971842
Wood, and articles of wood	Indonesia	301	New Zealand	320834318
	Canada	181	China	217468391
	Papua New Guinea	164	Indonesia	202735959
	United States of America	148	Malaysia	147228707
	Malaysia	121	USA	92654945
	Other	114	Germany	47331442
	Singapore	92	Chile	45200210
	China	62	France	41276732
	Thailand	59	Canada	40821830
	India	54	Czech Rep.	34258319

countries with no transport), but that increasing transport volumes did not increase ant interception frequency, when accounting for the effect of trade volume and geographic distance (Tables 4, 5).

### Species patterns

Intercepted ants identified to species or “species group” consisted of a combination of Native (n = 19), Endemic (37), Introduced (17), and not established (31) spe-



**Figure 5.** Ant interceptions into Australia by commodity group by year, for the top four commodity groups on which ants were found.

**Table 4.** AIC for 10 best candidate Poisson GLMs predicting total number of ant interceptions by country. All possible model combinations of these predictors were tested (512 total models): models not shown had higher AIC.

Regression formula	AIC	ΔAIC	Akaike weights
Ant interceptions from country - Trade value to Australia + Weighted transport to Australia + Non-zero transport to AU + GDPpc + geographic distance + com6 + com7 + com8 + com44	11960.77	0	1.00
Ant interceptions from country - Trade value to Australia + Non-zero transport to AU + GDPpc + geographic distance + com6 + com7 + com8 + com44	12018.95	58.18	10 <sup>-13</sup>
Ant interceptions from country - Trade value to Australia + Weighted transport to Australia + Non-zero transport to AU + GDPpc + geographic distance + com6 + com7 + com8	12138.72	177.94	10 <sup>-39</sup>
Ant interceptions from country - Trade value to Australia + Non-zero transport to AU + GDPpc + geographic distance+ com6 + com7 + com8	12169.69	208.92	10 <sup>-46</sup>
Ant interceptions from country - Trade value to Australia + Weighted transport to Australia + Non-zero transport to AU + GDPpc + geographic distance+ com6 + com8 + com44	12442.87	482.10	10 <sup>-105</sup>
Ant interceptions from country - Trade value to Australia + Weighted transport to Australia + Non-zero transport to AU + GDPpc + geographic distance+ com6 + com8	12489.41	528.63	10 <sup>-115</sup>
Ant interceptions from country - Trade value to Australia + Weighted transport to Australia + Non-zero transport to AU + GDPpc + geographic distance+ com7 + com8 + com44	12525.92	565.15	10 <sup>-123</sup>
Ant interceptions from country - Trade value to Australia + Non-zero transport to AU + GDPpc + geographic distance+ com7 + com8 + com44	12562.29	601.51	10 <sup>-131</sup>
Ant interceptions from country - Trade value to Australia + Weighted transport to Australia + Non-zero transport to AU + GDPpc + geographic distance+ com7 + com8	12694.84	734.06	10 <sup>-160</sup>
Ant interceptions from country - Trade value to Australia + Non-zero transport to AU + GDPpc + geographic distance+ com7 + com8	12767.64	806.86	10 <sup>-176</sup>

cies (Suppl. material 1: Table S1). Many identified species had only 1–3 interceptions however the species with the most records were those classified as introduced; 82% of interceptions identified to species level were of introduced species, with eight species having at least 100 interception records (Table 6). Most records were for the introduced *Paratrechina longicornis* (802 records). *Iridomyrmex purpureus* was the most

**Table 5.** Coefficients and standardized coefficients for the chosen model. Parameters com6, com7, com8, and com44 denote the total value of imports from the country into Australia of the given commodities: com6 for ‘Live plants, cut flowers, etc.’, com7 for ‘Edible vegetables’, com8 for ‘Edible fruit’, com44 for ‘Wood products’.

Parameter	Coefficient	Standardised coefficient	Standard error of standardised coefficient	95% confidence interval for standardised coefficient
(Intercept)	5.0668	1.3349	0.0440	(1.249, 1.421)
Trade value	$6.9283 \times 10^{-11}$	0.2600	0.0062	(0.2478, 0.2722)
Weighted transport	$-2.3064 \times 10^{-6}$	-0.0843	0.0108	(-0.1055, -0.06324)
Non-zero transport indicator*	2.0912	2.0912	0.0423	(2.008, 2.174)
GDP per capita	$1.0379 \times 10^{-7}$	0.0019	0.0141	(-0.02569, 0.02949)
Geographic distance	$-3.2222 \times 10^{-7}$	-1.3275	0.0142	(-1.355, -1.3)
Live trees, plants, etc. trade value (com6)	$1.0563 \times 10^{-7}$	0.1650	0.0065	(0.1523, 0.1778)
Edible vegetables etc. trade value (com7)	$-2.3888 \times 10^{-8}$	-0.1844	0.0086	(-0.2012, -0.1676)
Edible fruits, nuts, etc. trade value (com8)	$1.3141 \times 10^{-8}$	0.1809	0.005	(0.171, 0.1907)
Wood and wood articles trade value (com44)	$2.0587 \times 10^{-9}$	0.0699	0.0051	(0.06004, 0.07989)

\* Note that this is a binary indicator variable, and is therefore not standardized.

**Table 6.** The number of records for the most commonly intercepted species separated by status (endemic, native, introduced, or not established) and whether the port of origin for the record was within (“yes”) or outside (“no”) the known range of the species.

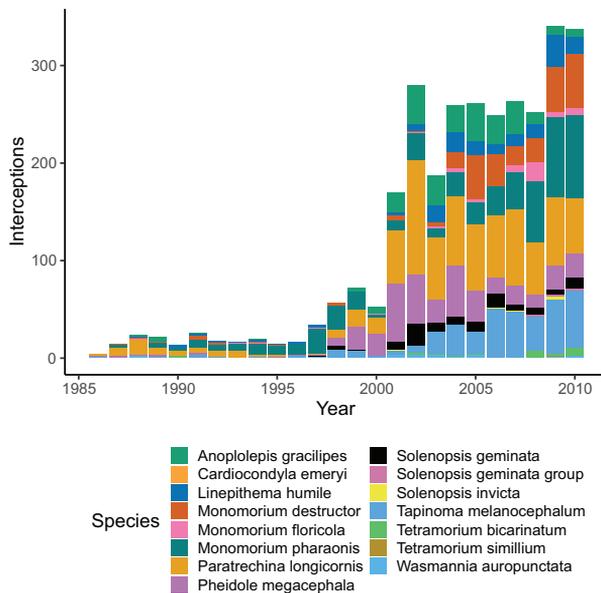
Status	Port of origin within known range		
	yes	no	unknown
<b>Endemic species</b>			
<i>Camponotus consobrinus</i>	3	19	7
<i>Iridomyrmex chasei</i>	1	12	1
<i>Iridomyrmex purpureus</i>	40	80	14
<i>Rhytidoponera metallica</i>	2	13	6
<b>Native Species</b>			
<i>Camponotus novaehollandiae</i>	7	16	5
<i>Nylanderia obscura</i>	12	1	2
<i>Ochetellus glaber</i>	81	6	7
<i>Oecophylla smaragdina</i>	29	6	9
<b>Introduced Species</b>			
<i>Anoplolepis gracilipes</i>	161	73	33
<i>Linepithema humile</i>	19	139	13
<i>Trichomyrmex destructor</i>	249	0	14
<i>Monomorium floricola</i>	54	0	1
<i>Monomorium pharaonis</i>	497	0	27
<i>Paratrechina longicornis</i>	703	0	99
<i>Pheidole megacephala</i>	364	0	22
<i>Solenopsis geminata</i>	25	77	11
<i>Tapinoma melanocephalum</i>	327	0	32
<i>Technomyrmex albipes</i>	94	0	3
<i>Tetramorium bicarinatum</i>	24	0	5
<i>Wasmannia auropunctata</i>	20	2	1
<b>Not Established Species</b>			
<i>Camponotus modoc</i>	16	0	0
<i>Camponotus pennsylvanicus</i>	57	4	2

commonly intercepted species in either the native or endemic categories with more than 95 records.

Interceptions of species classified as introduced increased with time, not levelling off like overall interception records did (Figure 1c; Figure 6). This was primarily driv-

en by increases in interceptions of *Tapinoma melanocephalum*, *Pheidole megacephala*, *Paratrechina longicornis*, *Monomorium pharaonis*, *Trichomyrmex destructor*, *Linepithema humile*, and *Anoplolepis gracilipes*, each of which had low levels of interceptions in the 1980s–1990s, increasing to higher levels through the 2000s (though interceptions of *Anoplolepis gracilipes* decreased 2008–2010)(Figure 6). Diversity had an initial peak around 1991, before decreasing until 1997. Diversity then increased until 2004, at which point it levelled off (Suppl. material 5: Figure S4).

The overall number of interceptions of not established species (those neither native to nor currently known to be established in Australia) remained low over time. Not established species were proportionally more likely to be detected on commerce originating from Africa or the Americas. For example, there were low levels of interceptions of the Nearctic species *Camponotus pennsylvanicus* throughout the whole range of years, and more sporadic low levels of other species. Records of endemic species (148 interceptions) are particularly remarkable, as these species are not found outside of Australia. Either these ants were transported away from Australia and then returned (unlikely), or the interception records are a product of at-border contamination (i.e., they were resident around the ports or airports in question, and moved on to cargo between arrival and quarantine processing). *Iridomyrmex purpureus* accounted for most endemic ant interception records, including almost all recorded endemic interceptions from 1986–1998 and more than half of total endemic interceptions from 2003–2006. Numerous other species were also detected solely or primarily in the 2001–2010 decade, including *Camponotus consobrinus*, and *Rhytidoponera metallica*



**Figure 6.** The most common identified introduced species intercepted in Australia ports of entry from 1986–2010.

with many interceptions over multiple years. These endemic species were generally detected at multiple locations.

For records identified to species, we also compared the source location for each record to bioregions in which the species is known to exist, i.e., to determine if ants are intercepted as coming directly from their known existing ranges, or via some intermediate location where they are not yet known to exist (Table 6). First, we excluded those records in which the interception was from the Australasian region; as for native, endemic, and introduced species these species will all be coming from a bioregion in which they are known to exist (because they are present in Australia). After excluding these Australasian interceptions, 2724 records remained (c. 73% of records with known species). Of the three categories (excluding endemic for reasons mentioned above), c. 11% of records were of unknown origin, 73% were recorded as coming from a bioregion within their known range, and the remaining 16% from bioregions outside currently known ranges.

## **Discussion**

Ants inhabit a wide variety of ecosystems, acting as predators, scavengers and mutualists as well as playing important ecological roles as ecosystem engineers (Lach et al. 2010; Del Toro et al. 2012). As invasive species, ants therefore have the potential to be associated with considerable economic and environmental consequences. Attempts to eradicate introduced ants are accompanied by great costs and are rarely successful (Hoffmann et al. 2016). Strategies aimed at prevention, including identifying high risk pathways and sources for new invaders, are therefore a priority. In this study, we analyzed historic interceptions of ants entering Australia to uncover biogeographic patterns of arriving ants, and correlate these data to patterns of trade and commerce. Three main conclusions come from this analysis. First, that there is significant variation in the level of taxonomic identification of intercepted ants, both over time, by genera, and, most importantly, between different locations. Given that biosecurity is of national importance, variations in efficacy between different parts of the country should be of significant concern. Second, the number of ant interceptions from different countries are associated with total volumes of transport and trade, but are also associated with the transport of specific commodity groups. That is, the risk associated with different transport pathways is non-uniform. Finally, ant species are typically transported from locations where they are already established, but not necessarily from where they are native. This pattern suggests that introduced species are more likely to spread once established in key transportation hubs (Passera 1994; Bertelsmeier et al. 2018).

Most ant interceptions arrived from Asia and Oceania, consistent with transport patterns into Australia. The largest numbers of interceptions occurred in Sydney, Brisbane, and Melbourne, however, there were also substantial numbers of interceptions in other ports of entry. On a per-country level, the presence of direct transport to Australia and volume of total trade to Australia were positive predictors of the num-

ber of ant interceptions, with increases in geographic distance and per-capita GDP of the source country both decreasing the expected number of interceptions from a given country. All of these predictors make sense: high trade increases opportunities for transport events to occur, per capita GDP suggests that more affluent countries are less likely to transport ants, and distance suggests that increased journey time may decrease the likelihood of ant survival. Overall trends in ant interceptions did not increase along with trends of transport / trade into Australia through the same time period. This pattern suggests that either the number of ants being transported per voyage has changed, decreasing from 2004–2010, or that the proportion of ant transport events that are detected has changed (e.g., Eyre et al. 2018). There is some evidence for the latter; due to occurrence overseas of Bovine spongiform encephalopathy, there was an increase in biosecurity effort in 2000–2002 such that 100% of imported containers were checked externally (Adam Broadley pers. comm.). This may explain the increase in ant detections within this time period, and then a subsequent return to lower levels of container inspections (30%). However, it is not certain to what extent ant interceptions may also be increasing or decreasing beyond this effect due to biosecurity effort.

Ant interceptions into Australia were primarily associated with the transport of particular commodities, particularly plant and timber products, and edible vegetables and fruit. There was also a substantial number of interceptions associated with transport itself (e.g., on vessels, baggage, personal effects, or containers). The commodities with which ant interceptions were primarily associated were not those responsible for the greatest total volume of imports into Australia; as such, it is clear that some commodities are much more likely than others to be associated with the transport of ants. However, the transport of these commodities alone is insufficient to explain patterns of ant interception as there were examples of countries that export plant products to Australia but had few ant interceptions (e.g., the Netherlands and New Zealand). There are likely a number of contributing factors to this discrepancy. For example, countries vary in their biosecurity measures on exported goods, the diversity of their ant fauna, and the degree to which their ants are likely to associate with human commerce or tolerate variation in abiotic conditions. Any of these explanations would be plausible for why, for example, the Netherlands or New Zealand had large volumes of trade in live plants, cut flowers, or wood products, but few ant interceptions. The association of ant interceptions with plant and wood products is not unique to this study (see Suarez et al. 2005; Ward et al. 2006; Lee et al. 2019), and is also prevalent in other insect groups (Liebold et al. 2012). It is possible that the next wave of ant invaders will include species from genera that commonly nest in plants / wood (e.g., *Camponotus* and *Crematogaster*) (Lee et al. 2019). The number of transported, but not yet known to be established ant species, identified in this and other analyses of interception records suggest the potential number of future introduced species is likely very underestimated at present (Miravete et al. 2013).

The number of ant interceptions associated with the transport of edible vegetables from Fiji accounted for more than 15 times as many interceptions as the next country associated with edible vegetables (Thailand), and almost 10% of all ant interceptions in the data set. Many of the interceptions from Fiji were also associated with leaves,

primarily Taro leaves but also Cassava, Roselle, Amaranth, and Bele. Taro leaves are a feature of Fijian cuisine, and Taro is one of Fiji's primary exports, with Australia a key destination (McGregor et al. 2011). However, it is not entirely clear why there are so many ant interceptions associated with these leaves, and further investigation of this phenomenon would be a valuable avenue for future work. One possibility is that Taro is known to have many honeydew producing insect associates, both on leaves and tubers (Palaniswani and Peter 2008). These aphids, scales and other insects might increase ant association and nesting and may explain why ants are more frequently intercepted when Taro is inspected.

The majority of interceptions identified to the species level were of known introduced species, and the number of these interceptions increased over time. It is not clear if the number being transported are actually increasing, or if they are just more effectively identified than other species due to improvement in the identification of ants generally, or of these known invasive species in particular, by biosecurity officers. This variation in identification also occurred among ports of entry with proportionally more interceptions identified to species in Queensland, Darwin, and Perth. One possible explanation for this is the detection of red imported fire ants, *Solenopsis invicta*, in south east Queensland in 2001, and subsequent concern over possible further incursions meaning extra effort was put in to identifying ant interceptions in Queensland. Overall, the proportion of interceptions not identified beyond "ant" decreased to a low level by the early 2000s, with the proportion of ants identified to species level rather than genus increasing through 2000–2010, suggesting a possible increase in overall expertise at identification, or at least an increase in confidence when identifying particular highly-invasive species, which were those most frequently identified to the species level.

However, it is also possible that many species are mis-identified or similar species incorrectly lumped into a single taxon (e.g. *Technomyrmex*, *Ochetellus*, *Camponotus*). Mis-identifications could have significant biosecurity consequences including allowing species to enter without treatment if they are mistaken as either native to, or already established, in Australia.

Most of the native, introduced, and not established species interceptions originated from locations from within their known native range. However, ~16% of interceptions of non-native species originated from outside their native range including *Camponotus novaehollandiae*, *Linepithema humile*, and *Solenopsis geminata*. These three are widespread introduced species, and these interceptions are coming from previously established introduced populations, a process known as the bridgehead effect and likely very important in influencing the invasion dynamics of ants and other invasive species (Bertelsmeier et al. 2018). The tropical fire ant, *Solenopsis geminata*, provides an example of this process as genetic data revealed an initial invasion from Mexico to Manila followed by subsequent invasions throughout Southeast Asia from this port city (Gotzek et al. 2015). Identifying hub countries that act as sources of invasive species is essential for planning biosecurity management. However, for such planning to be effective, up to date information on the current range of invasive species is essential – requiring real-time international cooperation and data sharing.

## Conclusions

In this study, we investigated historic records of ant interceptions to determine trends relating to potential ant invasions, to elucidate key pathways and hotspots, and to determine the commodities presenting the highest risk of future ant invasions in Australia. Given the difficult nature of eradication, once an ant species is firmly established (Hoffmann et al. 2016), focusing on early detection and quarantine is key for reducing the establishment of new invasions. In addition, these ant interceptions can be considered a model system from which more general conclusions about the global transport of invasive species can be drawn. Caley et al. (2015) argued that interception records may not provide an early warning system for insect incursions, as their study found that most successful incursions were not even intercepted by border quarantine in Australia (see also Eschen et al. 2015 for Europe). However, for species that were known to have been intercepted, incursion probability was higher for those with higher interception rates (Caley et al. 2015), indicating that the role of transport and commerce in facilitating higher propagule pressure/interception rates of species is key to informing biosecurity risk management. Whether the number of interceptions per species in this study equates/relates to establishment risk would require further study. Finally, in addition to inspection on arrival, more effort needs to be placed on inspection in high-risk ports of departure. Implementing port-of-departure biosecurity measures can be very effective at reducing contamination of goods as evidenced in New Zealand where ant presence in containers dropped from 17% to less than 1% (Nendick et al. 2006; Hoffmann et al. 2017).

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## Supplementary material I

### **Table S1. Ant species (or species group), number of records and status of ants identified from interception records to Australia from 1986–2010**

Authors: Elissa L. Suhr, Dennis J. O'Dowd, Andrew V. Suarez, Phillip Cassey, Talia A. Wittmann, Joshua V. Ross, Robert C. Cope

Data type: species data

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Link: <https://doi.org/10.3897/neobiota.53.39463.suppl1>

## **Supplementary material 2**

### **Figure S1. Flights into Australia 1999–2012**

Authors: Elissa L. Suhr, Dennis J. O'Dowd, Andrew V. Suarez, Phillip Cassey, Talia A. Wittmann, Joshua V. Ross, Robert C. Cope

Data type: measurement

Explanation note: Data from OAG Aviation (<http://www.oag.com>).

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Link: <https://doi.org/10.3897/neobiota.53.39463.suppl2>

## **Supplementary material 3**

### **Figure S2. Ant interception records into Australia separated by transportation type (aircraft, sea vessel or other) and source bioregion**

Authors: Elissa L. Suhr, Dennis J. O'Dowd, Andrew V. Suarez, Phillip Cassey, Talia A. Wittmann, Joshua V. Ross, Robert C. Cope

Data type: biodiversity data

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Link: <https://doi.org/10.3897/neobiota.53.39463.suppl3>

## **Supplementary material 4**

### **Figure S3. Import value over time for the 4 commodities with the most associated ant interceptions**

Authors: Elissa L. Suhr, Dennis J. O'Dowd, Andrew V. Suarez, Phillip Cassey, Talia A. Wittmann, Joshua V. Ross, Robert C. Cope

Data type: biodiversity data

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## **Supplementary material 5**

### **Figure S4. Shannon diversity index calculated annually for interceptions identified to species from the “introduced” species category**

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# Native generalist consumers interact strongly with seeds of the invasive wild cucumber (*Echinocystis lobata*)

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## Abstract

When alien plant species arrive in a new environment, they develop novel interactions with native biota that can range from negative to positive. Determining the nature and strength of these interactions is integral to understanding why some aliens are suppressed and others become highly invasive pests. For introduced terrestrial plants, seed and seedling interactions with native biota are crucial, because most nascent populations start from seed. Herein, we explored interactions between native generalist rodent and bird consumers and seeds of the invasive wild cucumber *Echinocystis lobata* by conducting seed-offering experiments in Poland. We also evaluated how interspecific competition from native plants and intraspecific competition from clustering of *E. lobata* seed (clustering resembling consumer seed caching) affected survival of seedlings and young plants. Native consumers interacted strongly with *E. lobata* seeds, with rodents removing 98% of seeds from ground locations and birds removing 24% of elevated seeds. Camera and live traps indicated that striped field mice *Apodemus agrarius* were the predominant rodent removing seeds. Camera traps and visual observations indicated that great tits *Parus major* and European jays *Garrulus glandarius* were the primary bird species removing elevated seeds. While some level of seed removal was likely attributable to seed predation, as indicated by seed coat remains, we also observed evidence that rodents may cache *E. lobata* seeds and *Garrulus glandarius* are known to cache and disperse seeds. Monitoring of seedlings indicated that increasing cover of native plants and clustering of *E. lobata* seedlings both reduced survival of seedlings and young plants due to inter- and intraspecific competition, respectively. Hence, caching by generalist consumers may disperse *E. lobata* seeds, which are heavy and lack dispersal adaptations, but such caching may also reduce individual seedling survival rates. Fully understanding invasion success of the *E. lobata* will require evaluating the net effects of generalist consumers on its recruitment and dispersal.

**Keywords**

biotic resistance, corvid, competition, *Echinocystis lobata*, rodents, seedling survival, seed predation, seed dispersal

**Introduction**

Understanding why some introduced plant species become problematic invaders, while others are naturalised residents, is a primary question motivating invasion ecology (Pyšek et al. 2012; Pearson et al. 2018). Successful establishment of an invader indicates that abiotic conditions are at least sufficient to support its fundamental niche requirements (Hutchinson 1957), suggesting an important role of biotic factors in defining the invader's realised niche and ultimate success (Elton 1958; Maron and Vilà 2001; Levine et al. 2004). Many biotic components of the recipient community will establish a variety of novel interactions with the invader, which may range from strongly negative to strongly positive (Mack et al. 2000; Keane and Crawley 2002; Parker et al. 2006). Determining the nature and strength of these interactions is crucial for understanding invader success.

Most terrestrial plant invaders establish nascent populations from seed. Therefore, seed survival, dispersal and seedling establishment are particularly critical processes determining the success of newly introduced plants (Colautti et al. 2006; Kleunen et al. 2018). Accumulating evidence suggests that post-dispersal seed predation by generalist consumers is a strong ecological filter affecting recruitment of both native and introduced plants (Reader 1993; Larios et al. 2017; Maron et al. 2012). Mounting evidence indicates that rodent seed predators have particularly strong effects on the establishment success of introduced plants (Reader 1993; Pearson et al. 2011; Maron et al. 2012). Granivorous birds and ants may also influence introduced plant success (Nuñez et al. 2008; Pearson et al. 2014), but far less is known about how these interactions, mediated by these consumers, affect recruitment of alien or native plants.

Generalist consumers may function as effective seed predators when they destructively consume seeds (Janzen 1971), but they also act as seed dispersers when they cache seeds for later consumption that they fail to retrieve and consume (Vander Wall 1993; Gómez et al. 2018). In this capacity, some birds may serve as important agents of seed dispersal, particularly long-distance dispersal, for both native and exotic plants (Richardson et al. 2000; Myczko et al. 2014). Rodents may similarly disperse seed through caching behaviour, but the dispersal distances tend to be much shorter (Ribble 1992; Iida 1996). The overall importance of seed dispersal via caching is not well understood, because it is logistically challenging to locate cached seeds to determine seed fates (but see Xiao et al. 2015; Bogdziewicz et al. 2018; Wróbel and Zwolak 2019). Importantly, when forgotten caches do germinate, the benefits to germinating seedlings are not always clear, because caching can result in high seedling densities, high competition and low survival (Howe 1989; Lambers et al. 2002; Kurek et al. 2018). In short, mounting evidence suggests that generalist granivores play important roles in

plant establishment that strongly influence plant invasion, while the balance between seed destruction and dispersal is poorly understood, even for native plants.

Herein, we quantified interactions between native generalist rodent and bird consumers and seeds of the introduced *Echinocystis lobata* in Poland, where this plant is invasive. We conducted seed-offering studies to quantify seed removal rates for both consumer guilds and identified species removing seeds via trapping, remote cameras and visual observations. We also quantified survival of naturally occurring solitary and clustered seedlings to understand how interspecific competition from native plants and intraspecific competition of the sort that might arise from consumer seed caching behaviour might affect seedling survival.

## Methods

### Study area

The seed removal experiments were conducted in October and November 2016 in four study sites located in two study areas in the Wielkopolska province of Poland: Noteć 1 (53°03'N, 16°52'E), Noteć 2 (53°01'N, 16°54'E), Kanał Grabarski 1 (52°10'N, 16°28'E) and Kanał Grabarski 2 (52°08'N, 16°28'E). The distance between the Noteć and Kanał Grabarski study areas was 96 km. The distance between Noteć 1 and Noteć 2 was 3.4 km and that between Kanał Grabarski 1 and Kanał Grabarski 2 was 2.5 km. Each study site consisted of a strip of typical riparian vegetation  $\geq 1$  km where *Echinocystis lobata* occurred and that was characterised by *Alnus glutinosa*, *Anthriscus sylvestris*, *Bromus inermis*, *Calystegia sepium*, *Fraxinus excelsior*, *Galium aparine*, *Glyceria maxima*, *Humulus lupulus*, *Phragmites australis*, *Poa palustris*, *Sambucus nigra*, *Symphytum officinale* and *Urtica dioica*.

### Study species

*Echinocystis lobata* is native to central North America from the east coast to the Rocky Mountains where it is associated with a broad range of riparian habitats, including stream, river and lake side areas (Foster and Duke 1990). This species was introduced to Europe at the end of the nineteenth and the beginning of the twentieth century as an ornamental plant (Tokarska-Guzik 2005). *Echinocystis lobata* is listed as one of the 100 most invasive alien species threatening natural ecosystems in Europe (Nentwig 2009), where it invades riparian communities (e.g. rushes, riparian forests and nitrophilous habitats). *Echinocystis lobata* can overgrow native herbaceous plants and it competes as well with native vine species, such as *Calystegia sepium* and *Humulus lupulus* (Tokarska-Guzik 2005).

*Echinocystis lobata* is an annual vine in the gourd family (Cucurbitaceae). It produces fleshy fruits 2.5–5.0 cm long and 2.5–3.5 cm wide that are covered by spines.

We have observed little consumption of the fruits in the introduced range (LD and LM, pers. observ.). Typically, fruits produce four seeds that dehisce at the end of the growing season, with the dried fruit remaining attached to the plant and the seeds falling to the ground. Hence, the diaspores overwinter as seeds not as fruits and are thus exposed to seed predators.

The seeds of the species are large (mean seed mass = 0.33 g; length = 17 mm; width = 8 mm; Dylewski et al. 2018) and smooth, lacking any specific adaptations for dispersal. Recruitment mostly occurs as single individuals, but also appears as loose aggregations of 2–4 seedlings or dense clusters, ranging up to 31 seedlings (Fig. 1). Since fruits commonly produce 3–4 seeds, loose aggregations are attributed to entire fruits falling to the ground. Dense clusters of large numbers of seedlings suggest caching by vertebrates, though this is difficult to confirm.

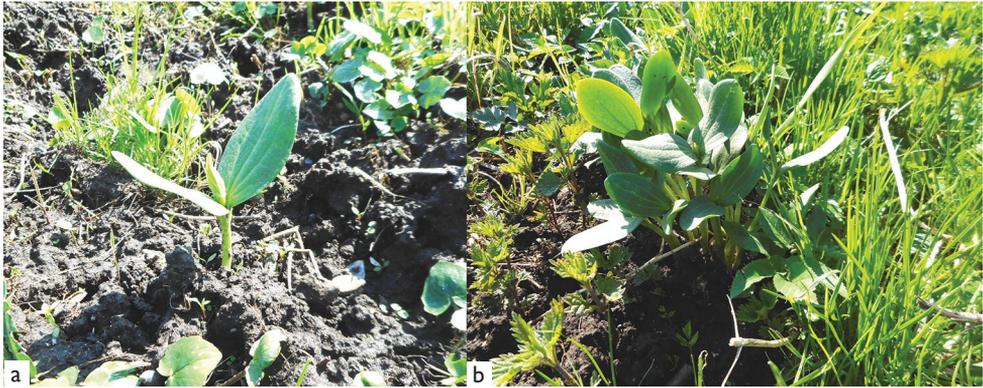
### Seed removal

We conducted seed removal experiments at ten stations at each study site, with each station separated by at least 100 m. We placed two green circular plastic trays (25 cm diam.) at each station (one for small rodents and one for birds) in the immediate vicinity of adult *E. lobata* individuals (< 1.5 m). The trays for small rodents were placed on the ground and covered with 4-cm wire mesh cages (30 cm × 30 cm × 15 cm height with two holes cut 8 cm × 8 cm) to minimise bird access to seeds (cameras observed no birds at these trays – see Results). The trays for birds were placed on top of wooden posts 1.5 m above the ground which protected seeds from mice and voles (cameras observed no voles or mice at these trays). We placed 20 *E. lobata* seeds in each tray (only seeds no fruits). We visited the trays each day for 5 days after initiation of the experiments to record the number of *E. lobata* seeds remaining.

To identify small rodent species potentially removing seeds, we established two live traps (TRIXIE TX-4192, size 5 cm × 5 cm × 17 cm) near each tray and at the central point between tray locations after seed removal experiments were complete. We trapped rodents for 24 h at each location four times over the course of the season, using *E. lobata* seeds as bait. During each visit, we surveyed each elevated tray from 30-m distance for 5 min to identify bird species removing seeds. Additionally, we set out two camera traps during the experiment at one randomly selected station at each study site, such that one camera was located next to a rodent tray and one next to a bird tray (four camera traps in Kanał Grabarski research area and four camera traps in Noteć research area).

### Seedling survival

To evaluate how seed dispersion and native plant abundance influenced seedling survival, we located thirty 2 m × 8 m plots containing naturally occurring seedlings (≥ 50 m apart) at two study sites (15 at Kanał Grabarski 1 and 15 at Kanał Grabarski 2)



**Figure 1.** Seedlings of *E. lobata* most commonly occur as **a** single individuals but also can be found in **b** tight clusters, suggesting seed caches.

to follow seedling fates over time. Our primary interest was to evaluate how single vs. clustered seedlings affected seedling survival in an effort to understand how caching activities might influence seedling success. Accordingly, each plot was selected to include one cluster of aggregated *E. lobata* seedlings and four isolated seedlings. Since *E. lobata* seedlings sometimes occur in aggregations of 3–4 due to seeds being collocated with fallen fruits, we focused on aggregations that contained five or more adjacent seedlings which could plausibly represent rodent caches (see Fig. 1a). Within each plot, we established one 1-m diameter circular subplot centred on each seedling and seedling cluster. Upon initiation of each subplot, we removed any additional *E. lobata* seedlings within the subplot and estimated cover of grasses, herbaceous plants and *Urtica dioica* therein. We visited each plot every 7 days (nine total visits per plot) and recorded survival of *E. lobata* seedlings and young plants.

### Statistical analysis

We applied survival analysis to examine seed removal using life tables (Kleinbaum 1996). In this analysis, survival is analogous to seeds remaining over the course of exposure to consumers (i.e. the probability of escaping seed removal) and is not intended to infer seed fate beyond removal. The interval for obtaining estimates was 5 days from the start to the end of the seed exposure period. To determine how the different factors affected the probability of escaping seed removal, we used Cox's proportional hazards model (Cox 1972) as applied by Myczko et al. (2014) to bird predation on acorns. First, we developed a full model examining effects of consumer (rodent or bird), study area (Noteć or Kanał Grabarski) and their interaction: consumer category  $\times$  study area on the probability of escaping seed removal (Table 1). As both factors and their interaction were significant and there is no option for post-hoc tests for the Cox's proportional hazard analysis, we followed up this analysis with two

**Table 1.** Results from Cox's proportional hazards analysis for measured factors and interactions potentially influencing the removal of wild cucumber seeds (n = 1600).

Factor	Parameter estimate ( $\beta$ )	SE ( $\beta$ )	$\chi^2$	P	95% CI
Vertebrate type	2.225	0.127	309.3	< 0.001	7.22–11.86
Study area	-0.711	0.163	19.0	< 0.001	0.36–0.68
Vertebrate type $\times$ Study area	0.865	0.179	23.5	< 0.001	1.67–3.37

models examining the effects of rodents and birds on the probability of escaping seed removal by study area separately.

We used the Mann-Whitney U test to compare the number of bird observations and rodent captures between research areas in order to relate consumer abundance to removal rates. We evaluated how seedling dispersion (clustered vs. single seedlings) related to survival of seedlings and young plants using Cox's proportional hazards model. In this model, we also evaluated how the different factors (i.e. seedling density (clustered or single), cover of grasses (dominated by *Phragmites australis*), cover of *Urtica dioica* and cover of other forbs and their interaction: seedling dispersion  $\times$  cover of grasses, seedling dispersion  $\times$  cover of other forbs), affected seedling survival (from the initial seedling phase through the young plant phase, up to 63 days). Finally, we used logistic regression as a further test of how seedling number within an aggregation affected the probability of seedling and young plant survival. All analyses were performed using IBM SPSS 21 for Windows (IBM SPSS 2012). All means reported  $\pm$  SE.

## Results

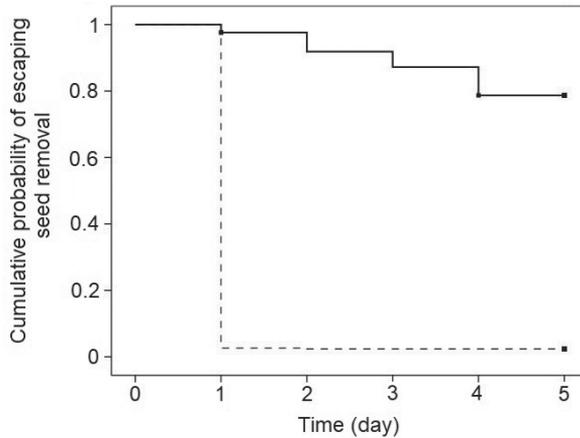
### Identification of seed removal agents

Numerous species were observed visiting and removing seeds from *E. lobata* seed offerings. We live-trapped four rodent species in the Noteć area: *Apodemus agrarius* (n = 95), *Myodes glareolus* (n = 6), *Apodemus flavicollis* (n = 3) and *Apodemus sylvaticus* (n = 2). In the Kanał Grabarski area, we live-trapped *Apodemus agrarius* (n = 57), *Myodes glareolus* (n = 26), *Apodemus flavicollis* (n = 2) and *Microtus arvalis* (n = 1). Cameras located at the ground trays generated 202  $\times$  30-sec. movies in the Kanał Grabarski area where we recorded *Apodemus agrarius* (n = 73), *Myodes glareolus* or *Microtus* sp. (n = 26) and *Apodemus flavicollis* or *Apodemus sylvaticus* (n = 11) visiting and/or removing seeds from trays.

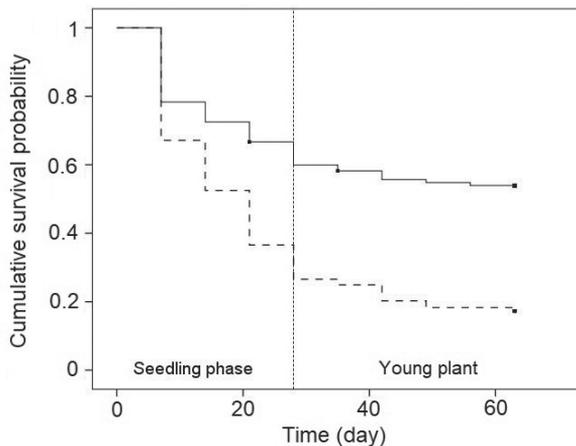
In the Noteć area, the cameras generated 268  $\times$  30-sec. movies documenting *Apodemus agrarius* (n = 139) and *Myodes glareolus* or *Microtus* sp. (n = 12) visiting and/or removing seeds from the trays. In both study areas, the cameras indicated that *Apodemus agrarius* were the predominant removers of *E. lobata* seeds. Camera traps at elevated seed trays generated 144  $\times$  30-sec. videos that identified two granivorous bird species removing seeds from trays: the omnivorous *Parus major* (n = 3) and the omnivorous, scatter-hoarding *Garrulus glandarius* (n = 2).

## Seed removal

The Cox's proportional hazard analysis for the full model was significant overall ( $\chi^2 = 710.9$ ,  $df = 3$ ,  $p < 0.001$ ), with consumer category ( $\beta = 2.22 \pm 0.13$ ,  $p < 0.001$ ), study area ( $\beta = -0.71 \pm 0.16$ ,  $p < 0.001$ ) and consumer category  $\times$  study area interaction ( $\beta = 0.87 \pm 0.18$ ,  $p < 0.001$ ), all significantly influencing the probability of removal of *E. lobata* seeds (Table 1). In the separate models, rodent effects did not differ between research areas ( $\chi^2 = 0.57$ ,  $df = 1$ ,  $p = 0.452$ ) but bird effects did ( $\chi^2 = 19.8$ ,  $df = 1$ ,  $p < 0.001$ ). The probability of seeds remaining at the end of the experiment was dramatically lower for seeds accessed by rodents ( $\bar{x} = 0.026 \pm 0.000$ ) than for seeds accessed by birds ( $\bar{x} = 0.862 \pm 0.005$ ), with 92.5% vs. 2.5% of seeds removed in 24 h by rodents versus birds, respectively (Fig. 2).



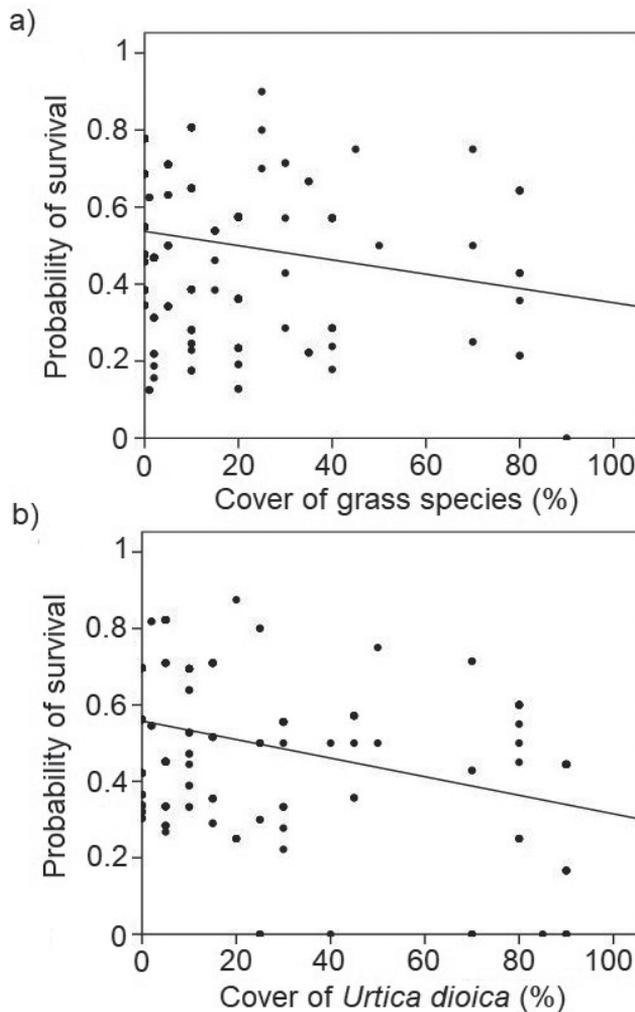
**Figure 2.** Probability of escaping seed removal (escape curves) for *E. lobata* seeds exposed to bird (solid line) and mammal granivores (dotted line).



**Figure 3.** Differences in survival probabilities for *E. lobata* seedlings and young plants for single (solid line) and aggregated seedlings (dotted line).

**Table 2.** Results from Cox's proportional hazards analysis of factors influencing the survival of wild cucumber seedlings and young plants (n = 421).

Factor	Parameter estimate ( $\beta$ )	SE ( $\beta$ )	$\chi^2$	P	95% CI
Seedling density	0.902	0.156	33.4	< 0.001	1.82–3.35
Cover of grass	-0.374	0.151	6.1	0.013	1.08–1.96
Cover of <i>Urtica dioica</i>	-0.443	0.169	6.9	0.009	1.12–2.17
Cover of other forbs	-0.289	0.200	2.1	0.149	0.90–1.98
Seedling density $\times$ Cover of grass	-0.231	0.169	1.9	0.172	0.57–1.11
Seedling density $\times$ Cover of <i>Urtica dioica</i>	-0.328	0.181	3.3	0.070	0.51–1.03
Seedling density $\times$ Cover of other forbs	-0.294	0.215	1.6	0.173	0.49–1.14



**Figure 4.** The effects of **a** cover of grass species and **b** cover of *Urtica dioica* on *E. lobata* seedling and young plant survival probabilities.

These results suggest that rodents may be the primary seed removing species. The mean removal rate for seeds over the 5-day period was higher for Noteć ( $\bar{x} = 0.907 \pm 0.005$ ;  $n = 400$ ) than for Kanał Grabarski ( $\bar{x} = 0.804 \pm 0.009$ ;  $n = 400$ ). This difference was consistent with the finding of fewer bird observations in the Noteć research area ( $\bar{x} = 1.54 \pm 0.35$ ) compared with the Kanał Grabarski area ( $\bar{x} = 4.68 \pm 1.50$ ) (U Mann-Whitney  $Z = 2.03$ ,  $p = 0.042$ ), suggesting that avian activity levels were correlated with avian seed removal rates. Rodent captures did not differ between study areas (U Mann-Whitney  $Z = 0.315$ ,  $p = 0.752$ ).

### Seedling survival

The mean number of seedlings growing from clusters was  $10 \pm 4.09$  (min–max 5–31).

The Cox's proportional hazard model, including all factors, was significant overall ( $\chi^2 = 51.9$ ,  $df = 7$ ,  $p < 0.001$ ), with seedling density ( $\beta = 0.902 \pm 0.156$ , Fig. 3), cover of grass ( $\beta = -0.374 \pm 0.151$ ) and cover of *Urtica dioica* ( $\beta = -0.443 \pm 0.169$ ) significantly influencing *E. lobata* survival (Table 2, Fig. 4), but interactions were all non-significant ( $p > 0.05$ ) (Table 2). Hence, native grasses and *Urtica dioica* appeared to have the ability to reduce seedling survival. The probability of survival of individuals growing in aggregation was  $\bar{x} = 0.173 \pm 0.020$ , whereas the probability of survival of individuals growing separately was  $\bar{x} = 0.542 \pm 0.445$ . The logistic regression indicated that the number of seedlings in an aggregation negatively correlated with survival of *E. lobata* plants ( $\chi^2 = 6.0$ ,  $df = 1$ ,  $p = 0.014$ ). Collectively, these results suggest that higher densities of *E. lobata* seedlings, such as those arising from apparent seed caching, greatly reduced seedling survival.

### Discussion

The success of introduced plants can be strongly influenced by the novel interactions they develop with native species in the recipient range, particularly natural enemies (Keane and Crawley 2002). In this study, we documented strong interactions between native generalist rodent and bird consumers and the invasive *E. lobata*. Our seed-offering experiments demonstrated that, after only 5 days, rodents and birds had removed 97.6% and 23.8% of *E. lobata* seeds, respectively. While we could not determine the fate of removed seeds in our study, these high seed removal rates suggest that *E. lobata* seeds may experience high levels of consumption by generalist omnivores. However, we also observed evidence that rodents may cache *E. lobata* seeds and *Garrulus glandarius* are known to disperse seed via scatter hoarding, suggesting that these consumers may have both positive and negative effects on this introduced plant.

*Echinocystis lobata* seeds set out in ground depots were quickly depleted by consumers. Both live traps and camera traps suggested that the primary seed removers were rodents, particularly *Apodemus agrarius*. While birds could possibly have removed

some of these seeds, these depots were covered by wire cages to limit bird access and no birds were captured in live traps baited with cucumber seeds or observed by camera traps at these stations. *Apodemus agrarius* are formidable seed predators (Babińska-Werka 1981; Brown et al. 2007; Baraiibar et al. 2009) and empty seed coats located at the ground depots and trays suggested that many seeds were consumed on site. However, these mice may store the seeds in caches (Zhang et al. 2016) and we found dense clusters of *E. lobata* seedlings that were strongly suggestive of rodent seed caches, while *E. lobata* seedlings may sometimes be clustered due to fruits falling before seeds dehisce (see above). The prospective caches we observed were often comprised of 1–2 dozen seedlings emerging in tight bundles, suggesting aggregated burials (Fig. 1).

In following seedling clusters over time, we found that survival of clustered seedlings was much lower than that of the more commonly observed dispersed seedlings, indicative of natural seed dispersal for this plant. Furthermore, increasing seedling density within clusters was correlated with reduced survival, suggesting a role of intraspecific competition, consistent with McMurray et al. (1997). While some seeds removed by rodents may be cached and dispersed over short distances, the clustering associated with cached seeds may reduce individual seedling survival rates (Lambers et al. 2002; Lu et al. 2015; Kurek et al. 2018). Overall, *E. lobata* seeds located on the ground experienced very high removal rates, suggesting rodent seed predation.

In contrast to seeds located on the ground, *E. lobata* seeds set out on elevated trays experienced much lower removal rates. Nonetheless, seed removal at these trays was still substantial, with almost 25% of the seed removed in 5 days. Visual observations conducted at all of the trays and cameras placed at a subset of the trays identified *Parus major* and *Garrulus glandarius* as primary removers of these seeds – both species are important seed predators (Bossemma 1979; Sherry 1989). However, *Garrulus glandarius* are also scatter hoarders that serve as an important long-distance dispersal agent for several deciduous tree species (Bossemma 1979; Pons and Pausas 2007). This species may carry seeds (especially acorns and other nuts) from 250 m to 5 km to storage sites (Vander Wall 1990; Gómez 2003). In temperate forests, *Garrulus glandarius* are an important dispersal vector of invasive *Quercus rubra*, contributing to its colonisation of new areas (Myczko et al. 2014). Our results suggest that birds may act as seed predators and also possibly important dispersers of *E. lobata* seed in the invaded region in Poland.

Competition with native plants is another important source of biotic resistance to alien plant seedling establishment (MacDougall and Turkington 2005; Dylewski et al. 2017). In monitoring seedling survival, we found that higher cover of dominant grass species and *Urtica dioica* was linked to higher seedling mortality. As a vine, *E. lobata*'s success as an invasive species is contingent upon growing tall enough to overtop other plants. Therefore, locations with lower competition from native plants, combined with lower seed predation, may be most susceptible to *E. lobata* invasion.

Introduced plants may interact with native consumers in a variety of ways that can influence plant invasion and alter native consumer abundance and behaviour. Many studies have shown that native rodent, bird and insect consumers will remove the seeds of introduced plants (e.g. Folgarait and Sala 2002; Nuñez et al. 2008; Carrillo-Gavilán

et al. 2010; Pearson et al. 2014). Fewer studies have taken the next step to demonstrate that native seed predators can suppress the establishment of introduced plants (Reader 1993; Pearson et al. 2011; Maron et al. 2012; Connolly et al. 2014), while others have shown that native consumers may serve as important dispersers that facilitate invasion (Lenda et al. 2012; Myczko et al. 2014; Wróbel and Zwolak 2019). Reciprocally, introduced plants may alter the abundance and behaviour of native consumers via food subsidies, apparent competition and habitat changes (Pearson and Fletcher 2008; Pearson 2009; Mattos and Orrock 2010; Guiden and Orrock 2017).

Our results suggest that, within this system, native consumers may both strongly reduce *E. lobata* seed availability, which could reduce local *E. lobata* densities and also facilitate its dispersal to new locations. In turn, the high production of large, palatable seeds could subsidise native consumer populations with a range of indirect effects. While our seed-offering studies were not designed to determine the outcomes of these interactions and our sampling was limited in space and time, our results do suggest that strong interactions are taking place between this annual invader and native consumers that could influence both *E. lobata* invasion and its effects on native communities within its introduced range in Poland.

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# A preliminary field trial to compare control techniques for invasive *Berberis aquifolium* in Belgian coastal dunes

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## Abstract

Non-native *Berberis aquifolium* is an invasive species in Belgian coastal dunes. With its strong clonal growth through suckers, this evergreen shrub outcompetes native species and affects dune succession. To prevent further secondary spread and mitigate its impact, there was an urgent need for knowledge on the effectiveness of control measures, both at the plant and habitat level. Here, we report on a first control experiment. Individual *B. aquifolium* clones were subjected to one of four treatments (manual uprooting, foliar herbicide application, stem cutting followed by herbicide or salt application), with regrowth being measured up to one year after treatment. We analyzed the relationship between kill rate, treatment, dune area, plant volume and number of plant stems using a generalized linear model. *Berberis aquifolium* plants proved most susceptible to foliar herbicide application (5% glyphosate solution), resulting in 88% (64%–97%) of the clones dying after treatment. The predicted kill rate decreased with an increasing number of stems under all treatments. We discuss the limitations of our experiment and the potential for actual field application of the different treatments. We present some guidelines for future control that may become further refined as experience builds up and we provide some recommendations for tackling invasive alien species in Atlantic dune ecosystems.

## Keywords

control, glyphosate, invasive alien species, Mahonia, management, oregon-grape, removal, shrub

## Introduction

The Belgian coastal dunes form a dynamic and diverse ecosystem that is home to a large number of characteristic species, many of which are regionally threatened (Provoost and Bonte 2004). Embryonic dunes, shifting white dunes, moss dunes, dune grasslands and dune slacks are considered high conservation value habitats of European importance (European Commission 2013). However, the dunes are highly fragmented, making them susceptible to external influences (Zwaenepoel 2009). Invasive non-native plant species are considered one of the most important threats to their biodiversity. The amount of non-native flora within the area has increased from about 5 to 20% since the 1970's, mainly representing garden escapes (Rappé et al. 1996; Provoost et al. 2010). Non-native shrubs and trees in particular are considered problematic invaders for the open habitats of the dune ecosystem (Table 1). All of these established species were deliberately introduced for ornamental purposes and are found as escapes from gardens, garden waste dumps or public plantings (Verloove 2006). Current populations of Oregon-grape *Berberis aquifolium* Pursh. (Berberidaceae), formerly known as *Mahonia aquifolium* Nutt. (Adhikari et al. 2015), in the Belgian coastal dunes are evergreen shrubs with pinnate leaves, yellow flowers and fleshy fruits. Plants can grow up to two meters, are many-stemmed and stoloniferous. The exact origin and taxonomy of most individuals in the wild is uncertain. Many of the invasive *Berberis* populations in central Europe may have arisen from hybridized cultivars of *B. aquifolium* with *Berberis repens* Lindl. or *B. pinnata* Lag., which belong to the compound-leaved *Berberis* spp. and originate from North America (Adhikari et al. 2015). They have been subject to selection for ornamental purposes, e.g. for faster growth rate, reproductive versatility, stress tolerance, pathogen resistance and greater biomass production (Jäger and Werner 2005; Ross and Auge 2008; Ross et al. 2008). Indeed, Ross et al. (2008) showed that the majority of cultivars and invasive populations in Germany formed a gene pool different from the native species. Common garden experiments showed that plants from invasive populations in central Europe grew larger in terms of stem length, number of leaves and above-ground biomass than either of the two native species (Ross 2009). Ross (2009) therefore concludes hybridization and subsequent selection by breeders have led to an evolutionary increase of plant vigor in the introduced range. The Belgian coastal populations are extensively suckering and have leaves with numerous leaflets which according to some authors suggests introgression from *B. repens* (Verloove 2018). However, Ross et al. (2008) could not find evidence of hybridization of *B. aquifolium* and *B. repens*. In this article, we will use the name *Berberis aquifolium* awaiting further evidence on the genetic identity of invasive coastal dune populations in Belgium but in order to clearly discriminate from native *B. vulgaris* L.

*Berberis aquifolium* is a successful neophyte that colonizes both natural and anthropogenic habitats and is found in a wide range of habitat types (grasslands, forests, coastal dunes ...) where it shows remarkable phenotypic plasticity (Ross et al. 2009). The species is shade tolerant and prefers dry to slightly moist, calcareous soils (Verloove 2006). It seems to show reduced vitality in completely sun-exposed

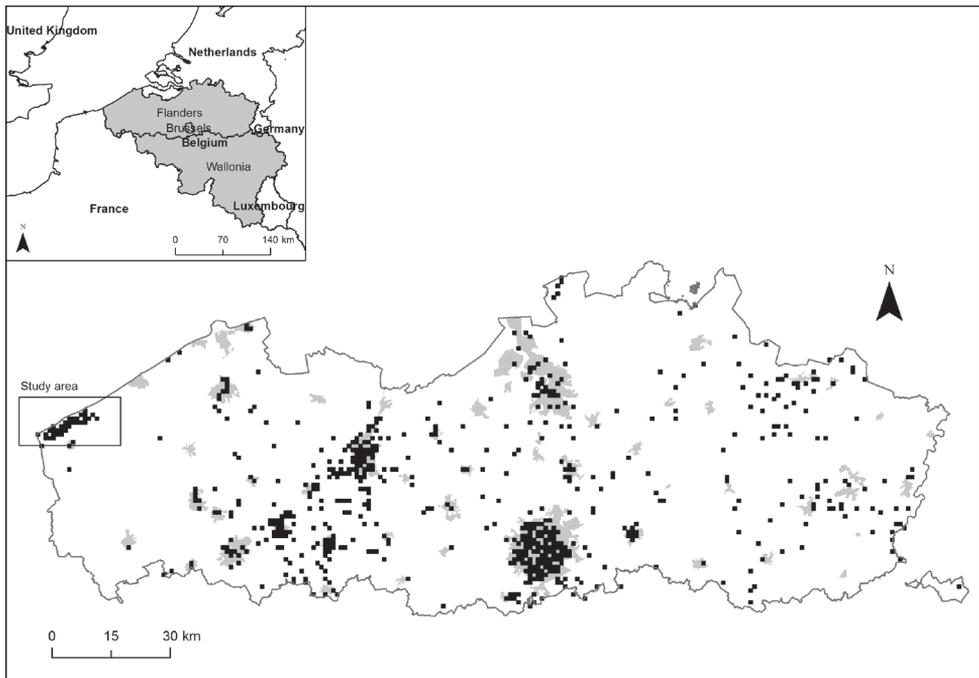
**Table 1.** Non-native tree, liana and shrub species established within the Belgian coastal dunes, in decreasing order of occurrence (% of dune areas and infected area in square meters based on field surveys in 46 nature reserves). *Populus alba*/*canescens* and *P. candicans* are frequently planted non-native tree species in the dunes but were not part of the survey. The Environmental Impact Assessment score for Belgium is added (ISEIA; Branquart 2007; Vanderhoeven et al. 2015). A = black list, B = watch list; 1 = isolated populations, 2 = restricted range, 3 = widespread in Belgium.

Species	Growth form	ISEIA	Surface area (m <sup>2</sup> )	% dune areas
<i>Rosa rugosa</i>	Shrub	B3	56757	63
<i>Berberis aquifolium</i>	Shrub	A2	34035	50
<i>Prunus serotina</i>	Tree	A3	5461	52
<i>Syringa vulgaris</i>	Shrub	–	4544	30
<i>Ribes odoratum</i>	Shrub	–	2986	11
<i>Symphoricarpos</i> spp.	Shrub	–	2874	26
<i>Robinia pseudoacacia</i>	Tree	–	1458	4
<i>Cotoneaster</i> spp.	Shrub	–	1392	41
<i>Lycium barbarum</i>	Shrub	–	420	15
<i>Ailanthus altissima</i>	Tree	A2	209	9
<i>Tamarix</i> spp.	Shrub	–	169	9
<i>Elaeagnus</i> spp.	Shrub	–	108	11
<i>Lonicera</i> spp.	Liana	–	106	13
<i>Prunus</i> spp.	Tree	–	88	13
<i>Parthenocissus</i> spp.	Liana	B3	83	4
<i>Ligustrum ovalifolium</i>	Shrub	–	72	9
<i>Ribes sanguineum</i>	Shrub	–	58	26
<i>Amelanchier</i> spp.	Shrub	–	44	9
<i>Yucca</i> spp.	Tree-like succulent	–	25	15
<i>Cornus</i> spp.	Shrub	–	15	24
<i>Baccharis halimifolia</i>	Shrub	A1	13	11
<i>Buddleja davidii</i>	Shrub	B3	7	7
<i>Euonymus japonica</i>	Shrub	–	5	2
<i>Pseudosasa japonica</i>	Shrub	–	4	2
<i>Rosa</i> spp.	Shrub	A3	4	4
<i>Quercus</i> spp.	Tree	–	3	4
<i>Sorbus</i> spp.	Tree	–	2	4
<i>Viburnum</i> spp.	Shrub	–	1	2

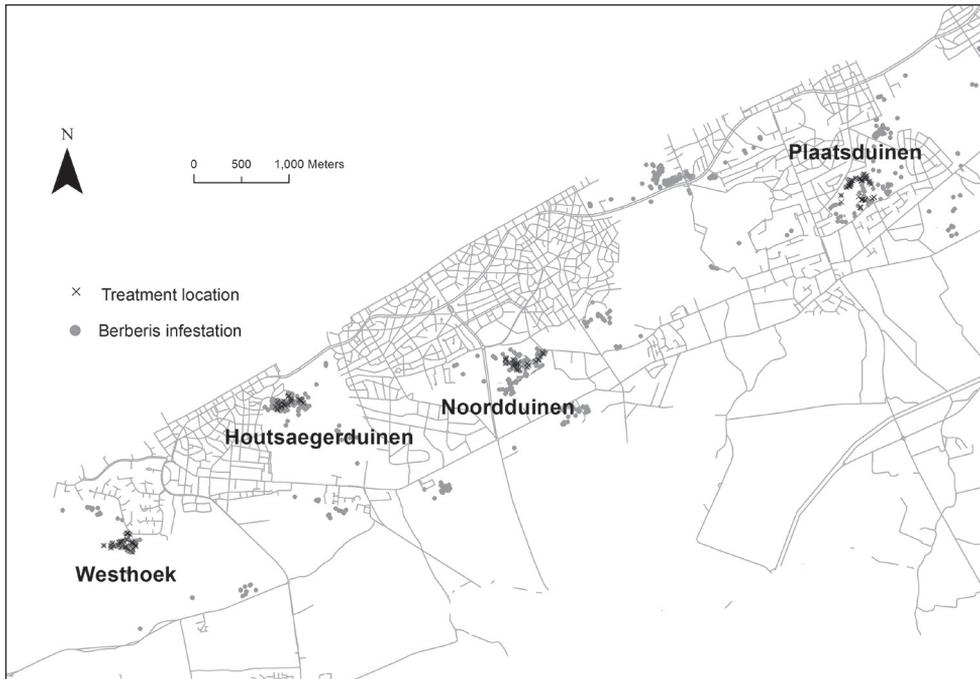
conditions (personal observation TA). In the Netherlands, *B. aquifolium* occurs in open dunes and dune woodland and is sometimes controlled in nature reserves (personal communication J. van Valkenburg). In central and eastern Germany, *B. aquifolium* is considered an aggressive invader and one of the most important invasive shrub species in forests (Auge and Brandl 1997; K. Schneider personal communication). In Switzerland, where it is present in the Jura, Plateau and south of the Alps, it invaded forests and ruderal sites (Wittenberg 2006). In Switzerland, there is also concern about the species contributing to higher abundance of the native fruit fly *Rhagoletis meigenii* Loew (Diptera: Tephritidae), a seed predator of the native shrub *Berberis vulgaris* L. This could lead to indirect effects on its original host, its parasitoids, and other hosts of those parasitoids (Soldaat and Auge 1998). In France, Germany and Belgium, it also occurs on calcicolous grasslands, which are a high conservation value habitat

in Europe (European Commission 2013). The recent expansion of the species or its hybrids/cultivars in Western Europe is possibly linked to global warming (Walther 2002). Generally, evergreen broad-leaved species of the laurophyllous plant functional type such as *B. julianae* Schneid., *Prunus laurocerasus* L., *Lonicera nitida* Wilson and *Cotoneaster* spp. can become more competitive as a lengthened growing season (to about 300–320 days without frost) releases them from climatic constraints on their establishment potential (Walther 2000, 2002; Keil and Loos 2005).

In Belgium, *B. aquifolium* was first recorded in the wild in 1906 and naturalized in the period 1920–1950 (Verloove 2002). Its distribution only increased rapidly since the 1990s; the reasons for this increase are unknown. The highest densities are found in the northern part of the country, notably in urban environments and in the coastal dunes (Verloove 2002, 2006; Van Landuyt et al. 2012; Fig. 1). The first observation along the coast dates back to 1972 (Van Landuyt et al. 2012). In urban areas it occupies a wide range of habitats such as waste land, disused industrial and railway yards, railway tracks and old walls. In the south of Belgium, it also occurs on rocky, wooded slopes (Verloove 2006). Provoost et al. (2010, 2015) mapped the distribution of non-native shrub and tree species in nature reserves along the Belgian coast using systematic surveys (Fig. 2). *Berberis aquifolium* was the most frequently encountered non-native shrub species, and the survey also indicated that gardens and public plantings bordering natural areas represent a major source of introduction (Provoost et al. 2015; Table 1).



**Figure 1.** Distribution of *Berberis aquifolium* in Flanders at a 1 km<sup>2</sup> scale (Van Landuyt et al. 2012) with an indication of the study area. Grey color indicates urban areas. Inset: location of Belgium and its three administrative regions (Flanders, Brussels, Wallonia) in northwest Europe.



**Figure 2.** Distribution of *Berberis aquifolium* (2007–2015) in the study area with management trial locations within four dune sites along the Belgian coast.

It grows in a wide variety of vegetation types ranging from moss dunes and dune grasslands to scrub and woodland. Along the Belgian coast, *B. aquifolium* has invaded gray dunes (Natura 2000 habitat code 2130 sensu European Commission (2013)), dunes with sea-buckthorn *Hippophae rhamnoides* L. (habitat code 2160), *Salix repens* L. communities (2170) and dune forests (2180). *Berberis aquifolium* plants in Belgian dunes commonly host the rust fungus *Cumminsia mirabilissima* (Peck) Nannf. but this does not seem to affect the species invasion (personal observations). The species' numerous blue berries (drupes) are easily dispersed over long distances by thrushes and other songbirds as was shown for other *Berberis* species (Silander and Klepeis 1999). Therefore, the plant can appear everywhere within the dune sites, including places which are hardly accessible to managers, such as under native *H. rhamnoides* shrub. *Berberis aquifolium* has rapid clonal growth, mostly by stem layering and below-ground stolons (Auge and Brandl 1997). Through its highly branched root system, the species attaches itself firmly in the sand. Manual pulling of mature plants can therefore be difficult and labor-intensive. As a result of its strong vegetative growth with root suckers, the species can locally appear in monospecific stands, overgrowing and displacing native species and thoroughly impacting dune succession (Verloove 2002, 2006). For instance, recent (2018) repeated vegetation mapping of the Noordduinen (Fig. 2) revealed that within 16 years, *B. aquifolium* managed to dominate and replace 2% of the indigenous scrub area (Sam Provoost, unpublished data). At the level of 50 × 50 m<sup>2</sup> squares, 28% of the Noordduinen is now infested with *B. aquifolium* and the species managed to dominate

and completely replace 2% of the native scrub area. As described for other scrub species in coastal dunes such as *Prunus serotina* Ehrh. in the Amsterdam Waterwork Dunes (Ehrenburg et al. 2008), a further exponential increase is expected. This perspective justifies rapid and thorough eradication. Although no specific studies exist, the impact of *B. aquifolium* in the Belgian coastal dunes is comparable to other invasive shrub and tree species for which impacts are well documented such as *P. serotina* (Ehrenburg et al. 2008), *Rosa rugosa* (Isermann 2008a, b) and *Ailanthus altissima* Swingle (Kowarik and Saumel 2007; Landenberger et al. 2007). These studies have shown negative effects on biodiversity, mainly by shading out herb-, moss- or lichen-dominated communities or by exhibiting allelopathy towards other plant species. Also, due to their higher productivity compared to native vegetation, they can alter topsoil chemical properties leading to abiotic and biotic homogenisation (Vanderhoeven et al. 2005; Dassonville et al. 2008). As a consequence, *B. aquifolium*, with *R. rugosa*, *P. serotina* and *A. altissima*, was categorized on a black list of invasive species with confirmed negative impact in Belgium following ecological impact assessment. This categorization was based on its high dispersal capacity, the colonization of high conservation value habitats by the species in Belgium, the potential for competition with native species, physical alteration of its habitat and impact on succession through accelerated colonization of open habitats by woody vegetation (Vanderhoeven et al. 2015; Table 1).

Considering the current level of infestation of the dunes (Table 1, Fig. 2), with the species being firmly established in a few publicly owned nature reserves, full eradication is considered the most appropriate management strategy. However, information on effectiveness of management measures for this species is scarce. Several projects concerning management of invasive plants in Belgian coastal dunes are planned, strengthening the need amongst conservation managers for information on effective management techniques. In practice, two main types of infestation can be considered: scattered individual shrub units (regarded as clones) on the one hand, and high density areas, almost entirely covered by *B. aquifolium* on the other. Both infestation types are present in the area and they require a different management approach. The removal of large surface areas of high density *B. aquifolium* requires landscape scale measures that often involve mechanical removal with heavy machinery. Here, we focus on a few realistically applicable manual techniques for removal of individual *B. aquifolium* clones. These clones occur scattered within the landscape and are often inaccessible to heavy machinery.

## Methods

### Treatment of individual plants/clones

Experimental treatment of a selection of individuals with limited clonal extension was set up in four heavily infested dune sites (Figs 1, 2; Table 2). Individual *B. aquifolium* plants selected for treatment were located with a hand-held Garmin Foretrex 401 GPS,

**Table 2.** Number of *Berberis aquifolium* plants treated per dune area. LEAF = glyphosate leaf treatment, DIG = manual uprooting, STUB = cut and paint glyphosate, SALT = cut and paint salt solution.

Site	Location	LEAF	DIG	STUB	SALT	Total
Westhoek	51°05'06"N, 2°33'47"E	8	9	9	7	33
Houtsaegerduinen	51°06'02"N, 2°36'10"E	7	7	5	5	24
Noordduinen	51°06'15"N, 2°37'48"E	6	7	7	5	25
Plaatsduinen	51°07'29"N, 2°41'11"E	10	10	8	8	36
<b>Total</b>		31	33	29	25	118

photographed and marked with a flag so as to easily relocate them in the field after treatment. Height (cm) and diameter (cm) were measured and the number of stems was counted to get an idea of the dimension of each individual or clone. Within the selected plants, clone diameter was 85 cm on average (minimum 10 cm, maximum 6 meter). In April/May 2013, plants were subjected to one of the following management treatments: (1) manual uprooting by digging with shovels (DIG), (2) leaf treatment using a spray bottle with a 5% Roundup Max (450g/l glyphosate) solution (LEAF), (3) stem treatment (cut and paint) with the same glyphosate solution (STUB) or (4) stem treatment with a saturated salt (NaCl) solution (SALT). Each ramet of a clone was treated similarly. We alternated treatments in sequence to different plants. When one plant received a given treatment, we moved to the next plant and applied the following different treatment, making sure plants were sufficiently far apart (minimally 10 meters) so as not to treat the same plant clone and making sure the most closely located plants got different treatments. The 5% glyphosate solution corresponds with the recommended concentration for cut stump treatment of *P. serotina* which is higher than the recommended concentration (1.5–2%) for leaf treatment (Agentschap voor Natuur en Bos 2013). The salt treatment is regarded as an environmentally friendly alternative because the used quantities of salt, considered on a m<sup>2</sup> basis, result in soil salt concentrations far below the natural values (Rozema et al. 1983). The direct effect of treatments was compared in terms of categories of stem regrowth (dead, limited regrowth, vigorous regrowth), after six months following treatment (November 2013) and after one year (May 2014). Glyphosate application was performed on rainless days with an outside air temperature below 25 °C. Since January 2015 the use of herbicides in Flanders has been banned in areas that belong to or are used for public services, areas located in drinking water protection zones, in a zone of six meters alongside surface water such as canals, waterways and ponds and on roadside verges (Decision of the Flemish Government laying down detailed rules for the reduction of pesticide use by public services of 19 December 2008). A derogation on this ban for invasive species removal is subjected to specific permits. For the purpose of this experiment we obtained permission from the competent Agency for Nature and Forest who was also the owner of the sites. In total, 127 clones were treated. Unfortunately, nine could not be retrieved, probably because flags were removed by site visitors. Nonetheless, the resulting 118 clones measured were more or less equally distributed over sites and treatments (Table 2, Fig. 2).

## Data analysis

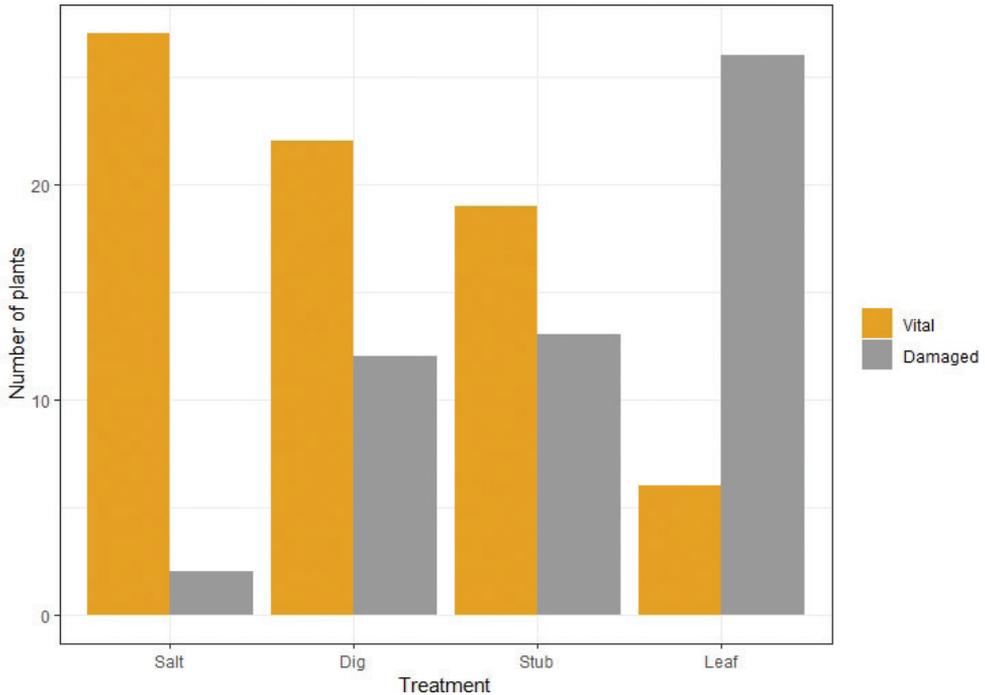
A minority of treated plants (8 out of 118) showed limited regrowth after one year. We therefore lumped limited and vigorous regrowth and considered those plants as vital after treatment. The product of plant diameter and plant height was used as a proxy for plant volume. We then investigated the relationship between kill rate (%), treatment (as a categorical variable with 4 values), dune area (as a categorical variable with 4 values), plant volume and number of stems using a generalized linear model with a binomial distribution and logit link (Quinn and Keough 2002) since we had a two-level response (dead or vital regrowth). We log transformed plant volume and number of stems to account for their skewed nature. We checked for correlations between factors in the model using Pearson's product-moment correlations (number of stems \* plant volume), Pearson's Chi-squared test (dune area \* treatment), Fisher's Exact Test for Count Data (regrowth \* dune area and regrowth \* treatment) and analysis of variance for plant volume and number of stems with dune area and treatment. We performed a multiple comparisons Tukey test to compare treatments, with a simultaneous p-value at 0.05. All analyses were performed in R version 3.1.2 (R Development Core Team 2014).

## Data resources

The data underpinning the analysis reported in this paper are deposited in the Dryad Data Repository at <https://doi.org/10.5061/dryad.zkh189361>.

## Results

Of the 118 treated plants, 45 were found dead, 8 exhibited limited regrowth and 65 were still found vital after treatment. Regrowth differed between treatments (Fisher's Exact,  $p < 0.001$ ) but not between dune areas (Fisher's Exact,  $p = 38$ ). Spraying *Berberis* foliage with herbicides clearly resulted in superior control with the majority of plants (26 out of 31) being killed. Salt treatment hardly affected regrowth as almost all plants (23 out of 25) remained vital after cut and paint with a salt solution. Digging (12 out of 33 killed) and stem treatment (13 out of 20 killed) showed intermediate kill rates (Fig. 3). There was an equal spread of treatments over dune areas (Pearson's Chi-squared test, Chi-square = 0.32633,  $df = 9$ ,  $p = 1$ ) and in every dune area plants with low and higher numbers of stems were treated. Regrowth was correlated with treatment (Fisher's Exact test for count data,  $p < 0.001$ ) but not with dune area (Fisher's Exact test for count data  $p = 0.38$ ). Number of stems and plant volume were only marginally correlated (Pearson's product-moment correlation  $t = 4.678$ ,  $df = 125$ ,  $r = 0.38$  (0.22–0.52),  $p < 0.001$ ). Treatment was not correlated with the number of stems (ANOVA:  $df = 3$ ,  $F = 1.36$ ,  $p = 0.26$ ) nor with plant volume (ANOVA:  $df = 3$ ,  $F = 2.15$ ,  $p = 0.10$ ). Number of stems was not correlated with dune area (ANOVA:  $df = 3$ ,  $F = 0.06$ ,  $p = 0.98$ ). However, plant volume was not independent of dune area (ANOVA:  $F = 8.52$ ,



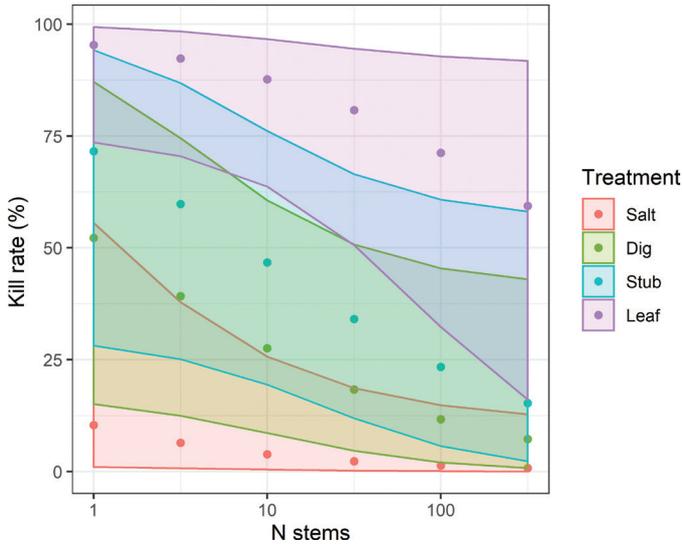
**Figure 3.** Number of vital and damaged *Berberis aquifolium* plants per treatment. Salt = cut and paint salt solution, Dig = manual uprooting, Stub = cut and paint glyphosate, Leaf = glyphosate leaf treatment.

$p < 0.001$ ), with the areas Plaatsduinen and Westhoek having bigger treated plants than Houtsaegerduinen and Noordduinen. We therefore included all factors in the generalized linear model but because of the collinearity between plant volume and area we need to be careful when interpreting effect sizes of these parameters. The predicted kill rate for *B. aquifolium* plants decreased with an increasing number of stems under all treatments. This decrease was most obvious for leaf treatment compared to other methods (Fig. 4). Leaf treatment of *B. aquifolium* resulted in superior control and was significantly different from all other treatments (Fig. 5). For leaf treatment the predicted average kill rate across all dune areas for the median amount of stems (10) and the mean log(volume) (5.2) was 88 % (95% CI: 64%–97%) (Fig. 5). The average kill rate was lower for the other treatments: 47% (19%–76%) for cut and paints and 28% (9–61%) for manual removal. Salt treatment had almost no effect with an average predicted kill rate of 4% (0.4%–26%) for salt treatment.

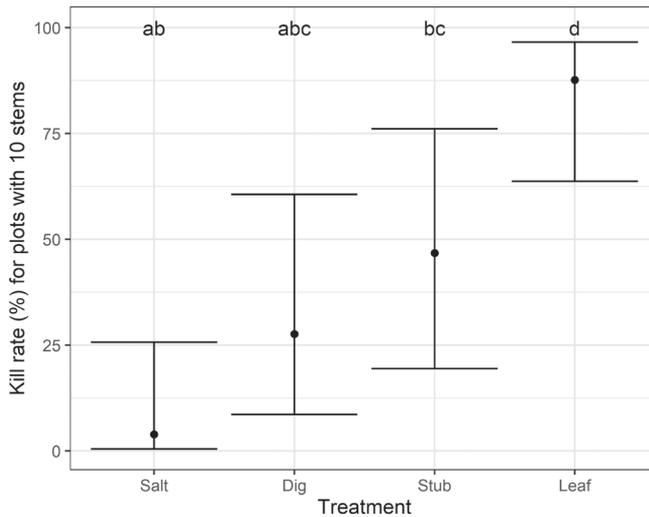
## Discussion

### Invasive species in Belgian dune ecosystems

Ornamental exotic species are increasingly causing problems for native biodiversity in Belgian coastal dunes. As the dunes are highly fragmented by urban development, the



**Figure 4.** Modelled kill rate as a function of the number of plant stems under different treatments. Salt = cut and paint salt solution, Dig = manual uprooting, Stub = cut and paint glyphosate, Leaf = glyphosate leaf treatment.



**Figure 5.** Modelled kill rate estimates (95% CI) for different treatments. Salt = cut and paint salt solution, Dig = manual uprooting, Stub = cut and paint glyphosate, Leaf = glyphosate leaf treatment. For ease of comparison we plotted the average predictions across all dune areas for the median amount of stems (10) and the mean log(volume) (5.2). Significant differences between treatments were tested with a post-hoc Tukey test. Treatments with the same letters above are not significantly different ( $p < 0.05$ ).

gardens surrounding the sites are the primary source of these plant species (Verloove 2002). However, because large populations of invasives have built up within the dunes, these currently most probably act as the major secondary seed source causing further ex-

**Table 3.** Model outcome of the generalized linear model.

Parameter	Est.	SE	P
TreatmentSalt	0.7197	1.8788	0.70168
TreatmentDig	2.8180	1.8148	0.12048
TreatmentStub	3.5178	1.9676	0.07381
TreatmentLeaf	5.3483	1.9388	0.00581***
RegionNoordduinen	0.6100	0.6856	0.37365
RegionPlaatsduinen	0.1144	0.7596	0.88033
RegionWesthoek	1.4214	0.7336	0.05268
log(Volume)	-0.6238	0.4031	0.12171
log(N_stems)	-0.9658	0.5616	0.08546

pansion (Fig. 2). Most of the problems caused by invasions are due to, at most, 15 shrub or tree species. Differences in growth form and ecology urge for a variety of removal techniques. Important characteristics for invasiveness are a high dispersal potential and the capacity for vegetative reproduction. *Berberis aquifolium* is among the worst in these respects, given its long-distance dispersal potential through frugivorous birds and its vegetative growth potential through root suckers. It has been widely planted for its ornamental value and functionality as an evergreen hedge plant. It shows vigorous vegetative growth and has a rooting system well adapted to the coastal dune environment. Therefore, the experience with *B. aquifolium* can be useful for a wider range of other invasive species such as *B. julianae* which has recently been observed in the Belgian dunes (pers. obs. WV).

### Conservation management application

Foliar application of a 5% glyphosate solution seemed by far the most effective way to remove isolated *B. aquifolium* clones, suggesting good uptake of the active compound through the stomata despite the species' glossy, leathery leaves. Manual uprooting of individuals is labor intensive and regrowth from thin root or stolon fragments is nearly inevitable. Cutting, even with glyphosate stem treatment, gives poor results and therefore seems inadequate. However, our experiments only included a single cut and we do not know the results of several years of mowing. Also, the results of the described experiment only give an impression of the aboveground regrowth after one year. Excavation of a number of individuals revealed that some roots did reshoot, even if the aboveground parts of the plant looked completely dead. Kill rates were therefore probably overestimated and retreating sites will probably be necessary in order to obtain complete removal or at least more accurate figures on the success of each treatment.

The results described here are in contrast with Stahl and Schwab (2014) who measured no effect of chemical treatment in invasive *B. aquifolium* populations of orchid-rich forest in central Germany. In this study, the herbicide used was Clinic (Nufarm) in a 33% glyphosate concentration which was applied in early July on the leaves with a paintbrush (Stahl and Schwab 2014). This higher concentration may have hindered effective uptake of the product by the leathery leaves or differences in environmental

circumstances (e.g. shade, soil conditions) might explain the contrasting results. In our study, we did not consider control plants as the emphasis was on establishing causal relationships between a set of treatments and an outcome and had hardly perceived any natural fall-out during field surveys prior to the experiment. Due to the lack of control plants, we can, however, not entirely rule out that kill rates of our glyphosate treatments would have been overestimated, although leaf-treated plants consistently turned brown within days after treatment. Furthermore, it should also be noted that the exact identity of invasive *Berberis* hybrids/cultivars in Belgian coastal dunes is unknown for the moment (see introduction). Ross et al. (2009) found no evidence for local adaptation of different populations in central Germany, but for the time being it cannot be ruled out that Belgian populations are of a different genetic constitution and therefore could react differently to management treatments.

Non-target effects of a proposed management method on the environment, economy or society are important to consider when deciding on management options for invasive species (Booy et al. 2017). This is especially true in nature reserves where the use of herbicides might impact other species of concern or may face public opposition. Since leaf application requires more glyphosate than the other control techniques, this method has more potential for non-target effects. However, visual inspections showed very little collateral damage around treated plants. This is logical as application on the leaf was performed with a hand sprayer on days with little wind and therefore was very precise. Moreover, non-target effects of the other chemical as well as mechanical treatments on non-target plant species, invertebrates and soil biodiversity cannot be ruled out and remain undocumented.

Optimizing the use of herbicides currently seems the most appropriate way to tackle *B. aquifolium*. The technique of leaf spraying is particularly useful in dunes which are inaccessible for heavy machinery, or in situations where mechanical removal using machines is inappropriate because of great conservation value or sensitivity of the local habitat. In our experiments, we followed the general advice for glyphosate application in *P. serotina* management. Herbicide treatments were performed on days without rain to prevent solution run-off from the leaves and on days with an outside air temperature below 25 °C to maximize the efficiency of the active compound glyphosate. Further experiments should be carried out, however, testing different types and concentrations of herbicide and optimal treatment timing and conditions. As *B. aquifolium* is an evergreen shrub and its flowers are conspicuous, it can easily be detected throughout the year. However, it often occurs under or in between native *Hippophae rhamnoides* where plants can be a lot harder to detect or (re)treat and where non-target effects of control are more difficult to prevent. Also, some of the dune areas are grazed by introduced cattle as a management technique. The effect of grazing on *B. aquifolium* is unknown although grazers can defoliate older plants. The choice of method should reflect on the characteristics of each site. For example, the experiments described here were performed in nature reserves and were therefore not allowed during the breeding season. Although potentially more cost effective and broadly applicable, chemical control might not be the preferred option everywhere. We acknowledge many questions

might need to be addressed before chemical control can become a viable option. For example, the use of herbicides is heavily restricted near areas used for drinking water extraction, several of which are located in the coastal dunes. Also, horizon scanning of new potentially effective compounds (e.g. triclopyr) and products (e.g. aquamaster, agridex) is often hindered by legal constraints.

As our experiment focused on individual shrubs, it offers prospects to more effectively control scattered clones which currently still represent a widespread type of infestation in Belgian dunes. However, in high density areas, almost entirely covered by *B. aquifolium*, a different management approach might be required for various reasons. The removal of large surface areas of high density *B. aquifolium* requires landscape scale measures that often involve mechanical removal with heavy machinery rather than manual removal. As a demonstration project, a heavily infested area with 100% *B. aquifolium* cover in the Noordduinen, was mechanically removed over a surface area of 350 m<sup>2</sup> in November 2013 using a 42 tons excavator equipped with a barred shovel aiming at sifting sand from plant material (Suppl. material 1). This was accompanied by intensive manual raking (approximately 9 man hours) which enabled the removal of most of the smaller remaining stolon fragments. Thickets and plant remains were removed using a tractor and trailer fitted with low pressure tires and using a fixed route in order to limit track formation and damage to adjacent areas. The site was revisited in May 2014 and the outcome measured in terms of *B. aquifolium* regrowth from different depths. The rooting system appeared to be relatively shallow (30–40 cm). The limited regrowth from superficially buried stolon fragments could easily be pulled out. This shows large patches of dense *B. aquifolium* can be removed mechanically. Several hundreds of square meters per day could be harvested, depending on the terrain conditions (relief) and soil moisture content. Even though the use of herbicides is strictly regulated in Flanders, and the potential for non-target effects was considered high with large-scale application, mechanical removal was preferred in this situation. Evidently, this method should fit the nature management goals of the area as it removes all vegetation and completely disturbs the soil profile. As very few native plant species can survive under the dense and evergreen *B. aquifolium* cover, botanical losses are generally limited. In our experiment, only *Rosa spinosissima* L. was of conservation concern and was also removed. Soil disturbance can also be seen as an opportunity for landscape-scale dune restoration. All over northwestern Europe, fixation and landscape senescence is seen as a threat to the specific biodiversity of coastal dunes (Arens and Geelen 2006, Provoost et al. 2011). As such, the removal of invasive plant species, and notably scrub, can be a lever for landscape rejuvenation and various management options are available (Day et al. 2003). Working in dry conditions is essential when performing this type of removal, as these facilitate the separation of soil fraction and plant material. Care should be taken to correctly dispose of the plant material which in this case involved a tractor with low tire pressure (Suppl. material 1). Manual aftercare on site and revisiting the sites during the next growing season is essential, as some regrowth of *B. aquifolium* from stolons cannot be ruled out. However, any remaining or new shoots mostly originated from superficially buried fragments and could easily be pulled out by

hand. It should be noted that only very limited regrowth was observed at the treated site six months after removal. This was still the case during field visits in summer 2015 (personal observation WVG) and is in sharp contrast with similar mechanical removals of other invasive plant species in Belgian dunes such as *Rosa rugosa* and *Ribes aureum* Lindl. which seem to have a higher potential to reshoot. The success of the mechanical scrub removal also contrasts with the manual digging treatments performed on individual clones, where large regrowth was observed. It is possible that the crane can dig up the entire root system while manual digging does not remove all of the roots.

The potential of *B. aquifolium* to become invasive in Belgian dunes was already predicted by Verloove (2002) who also advised quick removal at the time. Also, permanent monitoring of biodiversity in the Belgian coastal dunes revealed an urgent need to tackle invasive shrub invasions (Provoost et al. 2010, 2015). *Berberis aquifolium* is currently still confined to the western coastal dune areas (Fig. 2). In order to prevent its further spread to the eastern parts of the Belgian coastal dunes, removal of the current populations acting as sources of secondary spread is urgent. *Berberis aquifolium* is subject to risk communication and recommendations towards the general public as well as horticulture professionals within the framework of the Belgian Life+ project AlterIAS, in order to limit its use near habitats of high conservation value (Halford et al. 2014). This incentive should further be put into practice near coastal dunes in order to raise awareness with local horticultural stakeholders (garden centers, horticulturists, park managers etc.), public bodies and private owners to prevent the species from being planted and used in gardens and public greenery near coastal dune reserves. In parallel, the potential of promoting native alternatives such as *B. vulgaris* can be explored.

On 1 January 2015 Regulation 1143/2014 on the prevention of the introduction and spread of invasive alien species (IAS) entered into force which prohibits trade and possession of invasive species on a Union List and enforces surveillance, rapid eradication, prevention and management actions on them. However, with the exception of *B. halimifolia* L. and *A. altissima* Swingle, none of the problematic shrub and tree species mentioned in this study, nor detrimental dune invasives such as *Carpobrotus edulis* (L.) L. Bolus or *Acaena novae-zelandiae* Kirk, are on the current list of regulated species. The drafting of a list of IAS of regional concern for Atlantic dunes could be a good alternative to prevent establishment of invasive species detrimental to this unique ecosystem and to prioritize action on already established invasives. The drafting of such regional lists should be based on sound risk assessment methodologies (Gallardo et al. 2015; Roy et al. 2018) but the prioritization should also properly consider risk management options and their feasibility (Booy et al. 2017; Vanderhoeven et al. 2017).

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**Supplementary material I****Photos showing removal methods trialled on individual plant (clones) in this experiment**

Authors: Tim Adriaens, Pieter Verschelde, Emma Cartuyvels, Bram D'hondt, Edward Vercruysse, Wouter van Gompel, Evy Dewulf, Sam Provoost

Data type: multimedia

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# Stuck amongst introduced species: Trophic ecology reveals complex relationships between the critically endangered Niau kingfisher and introduced predators, competitors and prey

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## Abstract

The introduction of mammals on oceanic islands currently threatens or has caused the extinction of many endemic species. Cats and rats represent the major threat for 40 % of currently endangered island bird species. Direct (predation) and/or indirect (exploitative competition for food resource) trophic interactions are key mechanisms by which invaders cause the decrease or extinction of native populations. Here, we investigated both direct and indirect trophic interactions amongst four predator species (i.e. animals that hunt, kill and feed on other animals), including three introduced mammals (*Felis silvestris catus*, *Rattus rattus* and

*Rattus exulans*) and one critically endangered native bird, the Niau kingfisher (*Todiramphus gertrudae*). All four species' diets and prey availability were assessed from sampling at the six main kingfisher habitats on Niau Island during the breeding season. Diet analyses were conducted on 578 cat scats, 295 rat digestive tracts (218 *R. exulans* and 77 *R. rattus*) and 186 kingfisher pellets. Despite simultaneous use of morphological and PCR-based methods, no bird remains in cat and rat diet samples could be assigned to the Niau kingfisher, weakening the hypothesis of current intense predation pressure. However, we determined that Niau kingfishers mainly feed on introduced and/or cryptogenic prey and highlighted the potential for exploitative competition between this bird and both introduced rat species (for Dictyoptera, Coleoptera and Scincidae). We recommend removing the cats and both rat species, at least within kingfisher breeding and foraging areas (e.g. mechanical or chemical control, cat sterilisation, biosecurity reinforcement), to simultaneously decrease predation risk, increase key prey availability and boost kingfisher population dynamics.

### Keywords

Island bird conservation, Introduced mammals, Predation, Competition, *Todiramphus gertrudae*, *Felis silvestris catus*, *Rattus* spp.

### Introduction

Islands, which host almost 40% of the critically endangered species on Earth on less than 6% of its total land area, are particularly vulnerable to biological invasions and represent a global conservation priority (Tershy et al. 2015). The long history of anthropogenic transportation of alien species to islands worldwide, including those of East Polynesia (e.g. since 1200–1300 A.D. in Wilmshurst et al. 2011), has resulted in deleterious effects on native wildlife that has often evolved without defences against predators or competitors (Steadman 2006; Anderson 2009). Alien (i.e. introduced) species inevitably disturb the natural balance of island food webs by modifying trophic links. This may affect the demography and abundance of native (and introduced) species, with further cascading effects (Courchamp et al. 2003; White et al. 2006; David et al. 2017).

Interactions like predation and competition shape the structure and dynamics of food webs in communities (Chase et al. 2002) and are critical considerations in species conservation and management (e.g. Brown et al. 2014; Marshall et al. 2016). Direct predation (i.e. top-down effect) is the predominant mechanism by which invaders can dramatically decrease populations of native species or even cause their extinction (Doherty et al. 2016; David et al. 2017). Introduced cats and rats have been identified as a prime cause of more than half the extinctions of island bird species recorded over the last centuries and still represent the major threat to 40% of currently endangered island bird species (Doherty et al. 2016; McCreless et al. 2016). Exploitative competition is an indirect mechanism of introduced species impact, leading to numerous native population declines but less often to extinctions (Davis 2003; David et al. 2017). Usually, local species restrict their realised niche and/or shift their niche to sub-optimal habitats to reduce niche overlap with introduced species (Reitz and Trumble 2002; Rankin et al. 2018).

Better understanding the feeding ecology of a threatened endemic species through diet analysis is an essential step towards its long-term conservation and management

(e.g. Gooch et al. 2015; Resano-Mayor et al. 2016). Trophic analyses also reveal complex trophic interactions between sympatric introduced and native species, particularly relevant to islands simultaneously threatened by several introduced species. For example, predation rates can be estimated (e.g. Bonnaud et al. 2009) and the potential for exploitative competition or competitive exclusion evaluated by measuring trophic overlaps between species (e.g. Du Preez et al. 2017). However, when prey are numerous and not limiting, high diet overlap does not necessarily imply significant competition (Cupples et al. 2011). Diet studies evaluating the environmental availability of shared resources are also required, to better assess the consequences of trophic interactions (White et al. 2006).

Here, we focused on one of the most threatened bird species worldwide, the Critically Endangered (BirdLife International 2016) Niau kingfisher *Todiramphus gertrudae*, whose sole population is confined to the small and remote atoll of Niau (Tuamotu Archipelago, French Polynesia). This human-modified and inhabited island hosts a range of cryptogenic and introduced species, including three of the most significant invasive predators worldwide: the pacific rat *Rattus exulans*, the black rat *Rattus rattus* and the feral cat *Felis silvestris catus*. *Rattus exulans* was probably introduced from South East Asia during the Polynesian colonisation of the archipelago ca. AD 1200 (Wilmshurst et al. 2011), while *R. rattus* and *F. s. catus* were likely introduced with the late 18<sup>th</sup> century arrival of European settlers (Atkinson 1985; Duffy and Capece 2012). Recently, a demographic study (Kesler et al. 2012) suggested that Niau kingfisher population dynamics could be limited by strong predation by cats and rats at critical demographic stages. Competition for food resources with introduced predators has also been suspected of impacting bird survival (Gouni and Sanford 2003; Coulombe et al. 2011). However, insufficient data is available from which to construct a robust conservation strategy.

This study aimed to identify possible trophic interactions (namely, predation and exploitative competition) between three introduced mammals and the Niau kingfisher during its reproductive season. We analysed the diet of these four 'sympatric' species for shared or exclusive prey to (i) identify the principal prey in the Niau kingfisher diet, (ii) quantify direct predation by introduced predators on kingfishers and (iii) evaluate trophic overlaps and identify prey taxa potentially at risk from exploitative competition, based on estimated prey availability. Such detailed understanding of the multi-invaded island food web should provide useful input to future restoration and conservation strategies.

## Materials and methods

### Study site

Niau Atoll (16°9'15"S, 146°21'20.4"W) (Tuamotu Archipelago, French Polynesia, South Pacific Ocean) (Figure 1), probably colonised by humans over 1000 years ago and regularly visited since 1820 (Butaud 2007), has a current resident population of ca. 200. Niau Island measures 26 km<sup>2</sup> and culminates at 6 m above sea level (Andréfouët et al. 2005). Its climate is tropical and oceanic (Mueller-Dombois and Forsberg 1998).

The vegetation consists of three main formations: (i) feo forest, a dense forest growing on jagged fossilised limestone coral covering 1800 ha of the atoll, (ii) *Cocos nucifera* plantations covering 700 ha, of which only 10% is used for copra farming and (iii) wetlands dominated by shoreline purslane *Sesuvium portulacastrum* and Jamaica swamp sawgrass *Cladium mariscus* (Butaud 2007).

### **The Niau kingfisher *T. gertrudae***

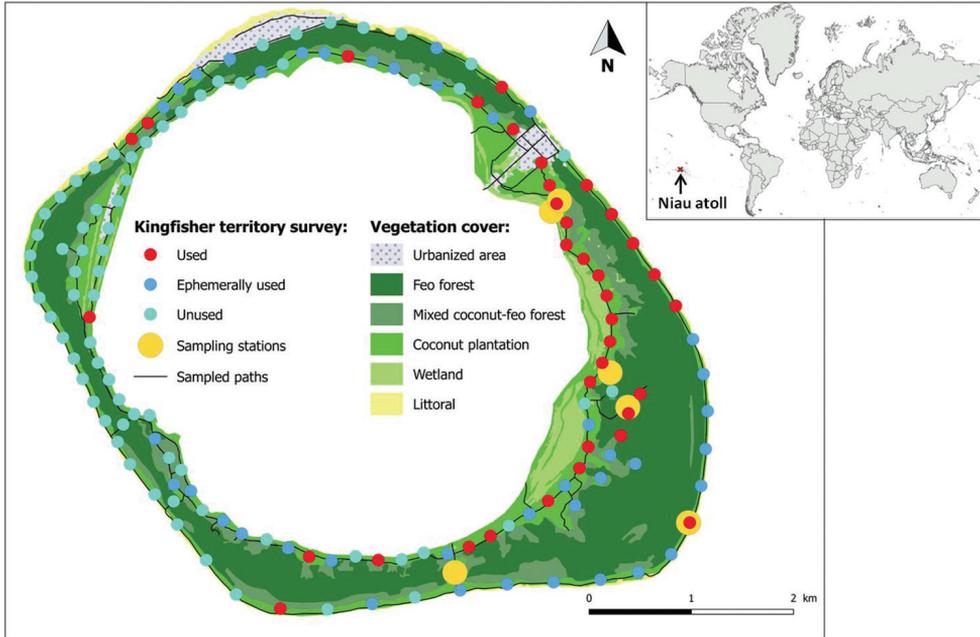
This endemic bird is confined to Niau and preferentially nests and forages within coconut groves located on the east side of the island, especially in semi-open and exploited groves (Gouni et al. 2006; Coulombe et al. 2011; Thibault and Cibois 2017) (Figure 1). Between 1970 and 1990, the Niau kingfisher was reported as common (Seitre and Seitre 1992), with a population estimated at 400–600 individuals (Holyoak and Thibault 1984). The reasons for its decline in the early 2000s remain unknown, but hypotheses include introduced mammalian predators (Kesler et al. 2012) and/or land-use changes (decreased coconut harvesting and use of fire to clear coconut plantations) (Coulombe et al. 2011; Thibault and Cibois 2017). Currently, the population has stabilised at ~140 individuals (100 mature individuals) (Thibault and Cibois 2017). To breed, the Niau kingfisher excavates the trunks of dead coconut trees, where it nests, tending to return to territories and partners over several years (Gouni and Sanford 2003; Holyoak and Thibault 1984). The breeding season lasts from October to February and both parents undertake parental duties until juveniles are independent (Kesler et al. 2012). To date, the few field observations available on the kingfisher's diet suggest that it mainly forages on terrestrial arthropods (Araneidea, Coleoptera, Dermaptera, Anisoptera) and lizards (Scincidae and Gekkonidae) (Holyoak and Thibault 1984; Marie 2006).

### **Predator diet analysis**

#### Sample types and collection

Sampling was conducted at two periods yearly over two consecutive years (from November 2009 to March 2011): the first in November at the beginning of kingfisher reproduction (laying, incubating and hatching periods) and the second in February–March at the end of the breeding season (fledgling and feeding periods of juveniles). Predators' diet samples and prey availability were sampled from six habitat types within surveyed kingfisher territories: two feo forests (low < 8 m and high > 8 m), three coconut plantations (abandoned, cultivated and intensively cultivated) and a wetland (Figure 1).

Rats were trapped in each habitat along a 320 m transect of 40 equally-spaced Victor (model BM201, Forest Stewardship Council, USA) snap-traps, set over five consecutive nights, baited with coconut flesh before dusk and checked early in the morning. Trapped rats were identified at the species level, weighed, sexed and dissected. Their



**Figure 1.** Map of Niau Atoll (Tuamotu Archipelago, French Polynesia). Location of the six sampling stations (rat diet samples and prey availability) and sampled paths (cat diet samples), surveyed Niau kingfisher territories (Coulombe et al. 2011) and main habitat types (Buraud 2007).

guts were collected and stored in 90% ethanol during transport and then frozen until examination. Cat scats were collected across all island paths, stored in Ziploc bags (SC Johnson, USA) and frozen until analysis. Kingfisher pellets were collected during the final field session (March 2011; end of reproductive season) below nests or hunting perches. The entire set of analysed diet samples consisted of 186 kingfisher pellets, 578 cat scats and 295 rat digestive tracts (218 *R. exulans* and 77 *R. rattus*).

To evaluate the availability of the main potential prey groups, the density of 16 different taxa (i.e. rats, Scincidae, Amphipoda, Isopoda and twelve terrestrial arthropod Orders) was estimated within the six studied habitats in February and November 2010 (see Suppl. material 1 for a detailed description of sampling protocols).

### Diet analysis

Morphological diet analyses were conducted on all the collected diet samples. Each rat's stomach contents and three last non-expelled faeces were individually extracted, homogenised and the entire volume analysed. Kingfisher pellets were dried before analysis and cat scats were analysed by washing over a 0.5 mm sieve under a stream of hot water. All hard prey remains (e.g. hairs, feathers, bones, scales, chitin) were isolated and identified to the finest taxonomic level possible under a dissecting microscope by comparison with reference materials from field-collected specimens and

via identification keys (for details, see Zarzoso-Lacoste et al. 2013, 2016). When possible, lizards were identified at the family level (Gekkonidae, Scincidae), arthropods at the order level (for Coleoptera, Hymenoptera, Orthoptera, Hemiptera, Dictyoptera, Dermaptera, Lepidoptera, Diptera, Odonata, Scorpiones, Araneae, Decapoda, Amphipoda and Isopoda) and Myriapoda at the subphylum level (containing Diplopoda and Chilopoda classes). Gastropoda and fish (Teleostei) were identified at the class and infra-class level, respectively. Birds were identified at the finest taxonomic level possible and assigned to the *Aves* class level in further statistical analyses. Mammalian prey corresponded only to the *Rattus* genus. Plants were only reported as presence/absence data for both omnivorous rat species.

To maximise the detection and identification of Niau kingfisher DNA in cat and rat diet samples, we implemented a PCR-based method (see Zarzoso-Lacoste et al. 2013, 2016) targeting the 5' end of the multi-copy mitochondrial cytochrome c oxidase subunit I gene (*Cox1*), a standard barcode sequence that enables species-level discrimination in birds (Hebert et al. 2004). This molecular method includes the combined use of three bird-specific primer pairs that were selected for their high power of detection, their specificity and their sensitivity towards bird DNA in cat and rat diet samples (for details, see: Zarzoso-Lacoste et al. 2016). This protocol notably deals with cases where bird soft tissue (e.g. vitellus, embryo or flesh) were consumed: our protocol is able to detect and identify bird DNA (including the DNA of the Niau kingfisher) at concentrations as low as  $0.01 \text{ ng}\cdot\mu\text{l}^{-1}$  (the lowest concentration tested in vitro using dilution series; see Zarzoso-Lacoste et al. 2016). Furthermore, we previously demonstrated that the molecular protocol systematically performs better in both the detection and the resolution of the taxonomic identification of birds in the diet samples of their predators than the morphological method (including samples where no hard remains of prey were found; see Zarzoso-Lacoste et al. 2016).

## Data analyses

All analyses were performed using the Statistical Software R version 3.5.1 (R Development Core Team 2018) – see Suppl. material 2 for the original data used to perform the following analyses.

## Diet descriptors

To describe each predator's overall diet and for each of the above prey taxa, several indices were calculated from (i) number of Prey Occurrences (*PO*) and (ii) Minimum Number of Individuals (*MNI*; White 1953). Prey Occurrence Frequency ( $POF = PO/S$ ) indicates the frequency of a prey category in predator diet samples, “*S*” being the total number of samples per predator. Mean Prey Number ( $MPN = MNI/S$ ) indicates the mean abundance of a prey category in predator diet samples. To more accurately evaluate the intensity of predator consumption on a particular prey category “*i*”, we

assessed (i)  $P_{iPN} = MNI_i / \sum MNI$ , the proportion of this prey relative to the total number of animal prey ingested by each predator and (ii)  $MPN_i = \sum (MNI_i / PO_j)$  reflecting the predation pressure on this prey. Finally, we constructed a bipartite network to visualise links between predators and their shared or exclusive prey, using *MNI* data and the *bipartite* R package (Dormann et al. 2008).

#### Diet comparison based on identified animal prey

We performed all subsequent analyses using *MNI* data for the 21 identified animal prey taxa (excluding plants and unidentifiable lizards and terrestrial arthropods). Abundance-based diet data were square-rooted prior to analysis to reduce the influence of the most abundant taxa (Clarke and Warwick 2001).

#### Sampling representativeness, diet richness and diversity

We used sample-size-based Hill numbers (orders  $q = 0, 1$  and  $2$ ), plus interpolated and extrapolated accumulation curves to estimate (i) the sampling representativeness of each predator diet and prey availability based on taxonomic richness ( $q = 0$ ) and (ii) predator diet diversity using the exponential Shannon's entropy index (giving more weight to rare species,  $q = 1$ ) and the inverse of Simpson's concentration index (giving more weight to abundant species,  $q = 2$ ) (Chao et al. 2014). We then compared their diet diversity up to the same base sample size (here,  $m = 1473$  prey *MNI*, see Chao et al. 2014 for more information), using the package *iNEXT* (Hsieh et al. 2016). Corresponding 95% confidence intervals were obtained by a bootstrap method based on 999 replications.

#### Identification of indicator prey in predators' diet

To identify the prey or combination of prey either included in the diet of a particular predator and/or contributing most to niche overlaps, we conducted "indicator species analyses" using the *multipatt* and *strassoc* functions of the *indicspecies* package (De Caceres and Legendre 2009). We used the *signassoc* function with 999 permutations and Sidak's correction for multiple comparisons to compare the abundance of prey taxa in predator diets.

#### Prey selection

We computed the Jacobs' electivity index ( $D$ ; Jacobs 1974) to investigate whether these four predators consumed prey proportionately to their relative densities in the environment. This index ranges between  $-1$  (negative selection) and  $+1$  (positive selection), indicating whether a prey species is selected, respectively, less or more than proportionate to its availability. We performed chi-square tests with Bonferroni adjustment to test for the significance of prey selection by the studied predators.

## Diet dissimilarity, breadth and overlap

We measured interspecific niche separation and intraspecific variability amongst predator diet samples using a Bray-Curtis dissimilarity matrix. We calculated the mean dissimilarity (*MD*) of diet composition between and within predator species using the *meandist* function of the *vegan* package (Oksanen et al. 2018). We then compared predator diets using a permutation-based test with the *betadisper* and *permutest* functions, corrected with Tukey's 'Honest Significant Difference' method for multiple comparisons. To visualise patterns in dietary dissimilarity within and amongst species in a low dimensional space, we performed Nonmetric Multidimensional Scaling (*NMDS*) using the *metaMDS* function. Finally, we calculated predator Diet Breadth (*DB*; from 0-specialised diet-to 1-generalist diet-) and Diet Overlap (*DO*; from 0 -no overlap- to 1 -complete overlap -), using the *nichevar* and *nicheoverlap* functions of the *indicspecies* package. Corresponding 95% confidence intervals were obtained by a bootstrap method based on 999 replications.

## Results

### Diet composition of studied predators

Kingfishers almost exclusively prey on terrestrial arthropods (*PiPN* = 50%; see Suppl. material 3), lizards (40%) and Decapoda (9%) (Figure 2[1]). The terrestrial arthropod taxa Coleoptera (23%), Hymenoptera (10%, of which 92% were Formicidae) and Dictyoptera (9%) were the most frequently and abundantly preyed upon. Scincidae were the most consumed lizards (23%) but Gekkonidae were also widely eaten (11%). Of these prey, Scincidae, Gekkonidae, Decapoda, Araneae and Coleoptera were strongly and significantly associated with the kingfisher diet (*component A* > 0.63) (Tables 1, 2).

The cat diet mainly consisted of rats (49%), followed by terrestrial arthropods (28%) and lizards (10%) (Figure 2[1], see Suppl. material 3). Dictyoptera were the terrestrial arthropods most frequently and abundantly consumed (8%), followed by Dermaptera (5%) and Coleoptera (2%). Gekkonidae were the most consumed lizards (4%). Rats and fish were significantly associated with cat diet samples (Tables 1, 2).

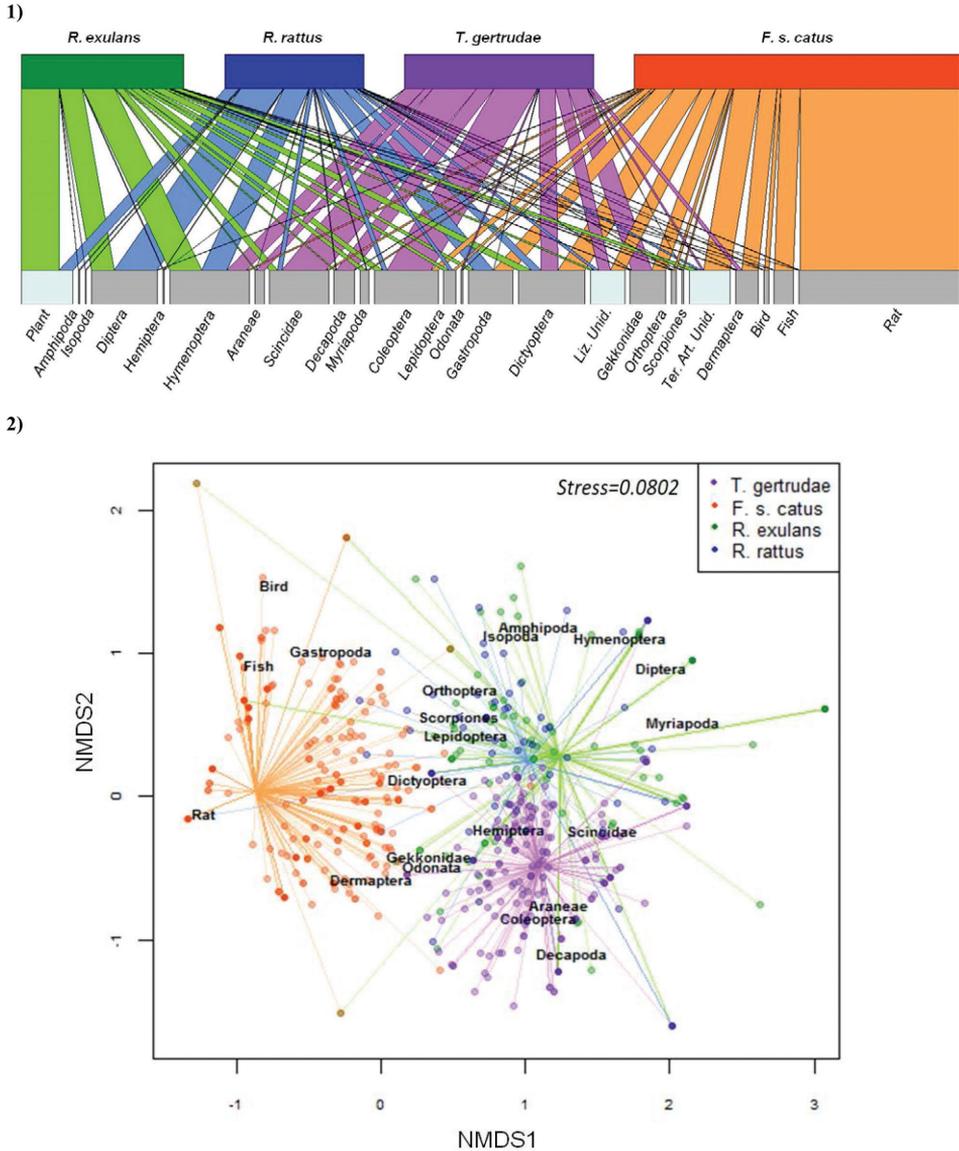
The rat diet was mainly plants (mainly coconut flesh, *POF* = 89% and 94% for *R. exulans* and *R. rattus*, respectively), but included a large proportion of animal prey (Figure 2[1], see Suppl. material 3). Terrestrial arthropods were the animal prey consumed most by both *R. exulans* and *R. rattus* (*PiPN* = 82% and 75%, respectively), followed by lizards (11% and 7%, respectively). *R. rattus* preyed more frequently on lizards, Gastropoda and crustacean than *R. exulans*. For both rats, Scincidae were the most consumed lizards (6% and 3% for *R. exulans* and *R. rattus*, respectively). Of the identified terrestrial arthropods, *R. exulans* and *R. rattus* mainly consumed Dictyoptera (11% and 8%, respectively), Hymenoptera (25% and 20%, respectively, all Formicidae), Diptera (11% and 8%, respectively, mainly larvae) and Coleoptera (7% and 4%, respectively). Hemiptera, Isopoda and Amphipoda were indicator prey particularly present in the *R. rattus* diet (Tables 1, 2).

**Table 1.** Prey as indicators of predators’ diet. Patterns and strength of the association between prey taxa and predators’ diet. *Component A*: probability that the surveyed predator belongs to the target predator group given the fact that the prey taxon has been found in the diet. *Component B*: probability of finding the prey taxon in diet samples belonging to the predator group.

	Prey	Component A	Component B	p value
<i>T. gertrudae</i>	Scincidae	0.69	0.80	< 0.001
	Gekkonidae	0.74	0.44	< 0.001
	Coleoptera	0.63	0.76	< 0.001
	Decapoda	0.82	0.44	< 0.001
	Araneae	0.81	0.17	< 0.001
	Odonata	0.88	0.02	0.05
	Scincidae + Coleoptera	0.88	0.61	< 0.001
<i>F. s. catus</i>	Rat	0.95	0.97	< 0.001
	Fish	0.87	0.17	< 0.001
<i>R. rattus</i>	Hemiptera	0.73	0.07	< 0.001
	Isopoda	0.87	0.03	0.01
	Amphipoda	0.73	0.03	0.03
<i>T. gertrudae</i> + <i>R. rattus</i>	Scincidae	0.84	0.66	< 0.001
	Coleoptera	0.82	0.63	< 0.001
<i>R. rattus</i> + <i>R. exulans</i>	Diptera	0.99	0.24	< 0.001
	Myriapoda	0.93	0.08	< 0.001
	Lepidoptera	0.89	0.06	0.01
	Orthoptera	0.85	0.06	0.01
<i>R. rattus</i> + <i>F. s. catus</i>	Gastropoda	0.87	0.14	< 0.001
<i>T. gertrudae</i> + <i>R. exulans</i> + <i>R. rattus</i>	Dictyoptera	0.86	0.40	< 0.001
	Hymenoptera	0.98	0.33	< 0.001

**Table 2.** Prey as indicators of predators’ diet. Test and comparison of the association between prey taxon and each predator diet. Values in bold highlight the predator that more significantly ( $< p_{adjusted}$ -Sidak-) consumed a particular prey than random.

	<i>T. gertrudae</i>	<i>F. s. catus</i>	<i>R. exulans</i>	<i>R. rattus</i>	$P_{adjusted}$
Gekkonidae	< <b>0.001</b>	1.00	1.00	1.00	0.004
Scincidae	< <b>0.001</b>	1.00	1.00	0.89	0.004
Coleoptera	< <b>0.001</b>	1.00	1.00	0.80	0.004
Araneae	< <b>0.001</b>	1.00	0.96	0.93	0.004
Decapoda	< <b>0.001</b>	1.00	1.00	0.97	0.004
Odonata	<b>0.01</b>	0.81	1.00	1.00	0.047
Rat	1.00	< <b>0.001</b>	1.00	1.00	0.004
Fish	1.00	< <b>0.001</b>	1.00	0.99	0.004
Bird	1.00	< <b>0.001</b>	0.77	0.57	0.008
Dermaptera	0.04	< <b>0.001</b>	0.99	0.95	0.004
Myriapoda	1.00	1.00	<b>0.01</b>	0.12	0.036
Hymenoptera	0.84	1.00	0.23	< <b>0.001</b>	0.004
Dictyoptera	0.35	1.00	0.82	< <b>0.001</b>	0.004
Gastropoda	1.00	0.33	0.99	< <b>0.001</b>	0.004
Orthoptera	1.00	0.96	0.26	<b>0.01</b>	0.047
Diptera	1.00	1.00	0.07	<b>0.01</b>	0.028
Hemiptera	0.84	1.00	0.69	<b>0.01</b>	0.020
Isopoda	1.00	1.00	0.46	<b>0.01</b>	0.032
Lepidoptera	1.00	0.98	0.18	<b>0.03</b>	0.129
Amphipoda	1.00	1.00	0.43	<b>0.06</b>	0.219
Scorpiones	0.76	0.50	0.46	<b>0.31</b>	0.771



**Figure 2.** Diet composition and overlap within and amongst the four studied predators. **1** Bipartite network. Lower boxes correspond to the identified (dark grey) and unidentified (light grey) prey taxa consumed by predators. Line and prey box widths show how frequently prey taxa are consumed by predators. Liz. Unid.: lizard unidentified, Terr. Art. Unid.: terrestrial arthropod unidentified. **2** nMDS of abundance-based Bray-Curtis dissimilarity of predator diet samples (solid dots). Solid lines represent the dispersion of a particular sample compared to the barycentre of its predator group.

Morphological and PCR-based methods, used in combination, allowed the detection of 28 bird individuals in cat ( $n = 20$ ) and rat ( $n = 3$  and  $5$  for *R. rattus* and *R. exulans*, respectively) diet samples and the identification of 24 of them as belonging to the

following seven species; *Gygis alba*, *Ptilinopus coralensis*, *Anous stolidus*, *Gallus gallus*, *Sterna bergii*, *Puffinus lherminieri* and *Accrocephalus atyphus* (for details, see Zarzoso-Lacoste et al. 2016). None was assigned to the Niau kingfisher.

### Sampling representativeness, diet richness and diversity

Rarefied and extrapolated species-accumulation curves (See Suppl. material 4) tended to saturate, indicating accurate sampling of predators' diet and mean prey availability in the environment. Diversity accumulation curves revealed that kingfishers presented the lowest prey richness ( $q_0D = 12.9$ ), although their prey abundance was more equally distributed amongst categories ( $q_1D = 6.9$  and  $q_2D = 5.8$ ) (See Suppl. material 4). Conversely, cats exhibited intermediate-level prey richness ( $q_0D = 17$ ), consuming prey categories more unevenly ( $q_1D = 5.3$  and  $q_2D = 2.9$ ). The two rat diets presented the highest prey richness ( $q_0D = 21.9$  and  $20.1$  for *R. rattus* and *R. exulans*, respectively), but differed in distribution of prey abundance ( $q_1D = 9.2$  and  $q_2D = 6.5$  for *R. exulans*,  $q_1D = 7$  and  $q_2D = 4.7$  for *R. rattus*).

### Diet dissimilarity and breadth

Mean dissimilarities (Table 3) and intra-species dispersion (Figure 2[2]) were highest in *R. rattus* and *R. exulans* diets (but lower for *R. rattus*), while intermediate values were observed for the Niau kingfisher, with the lowest values for cats. Differences were significant for all pairs of predators (permutation test,  $p_{adjusted} < 0.001$ -TukeyHSD-), except between *R. exulans* and *R. rattus* ( $p_{adjusted} = 0.13$ ) (Table 4). These results are consistent with calculated predator diet breadths (DB, see Suppl. material 3), the two rat species showing the widest diet breadth (0.43,  $CI = 0.42$ – $0.44$ ), closely followed by the kingfisher (0.42,  $CI = 0.41$ – $0.42$ ), while the cats showed the narrowest (0.35,  $CI = 0.33$ – $0.35$ ).

### Prey selection

Jacobs' electivity index ( $D$ ) confirmed that the kingfisher positively selected its main prey (i.e. Scincidae, Coleoptera, Hymenoptera, Dictyoptera and Dermaptera) (Table 5). The kingfisher negatively selected Araneae and strongly avoided almost all the other taxa. Of the main kingfisher prey, Dictyoptera and Dermaptera were strongly and positively selected by both rat species and cats ( $D > 0.92$ ,  $p < 0.001$ ; and  $D = 1$ ,  $p < 0.001$ , respectively). Scincidae, Coleoptera and Hymenoptera were also positively selected by *R. exulans* ( $D > 0.76$ ,  $p < 0.001$ ) and *R. rattus* ( $D > 0.62$ ,  $p < 0.001$ ), while cats slightly positively selected Coleoptera and tended to shun Hymenoptera ( $D = 0.48$  and  $-0.26$ , respectively,  $p_{adjusted} < 0.001$ ).

**Table 3.** Analysis of inter and intra species diet dissimilarities. Mean distance calculated based on the Bray-Curtis dissimilarity matrix between samples of each predator (diagonal) and between each pair of predators.

	<i>T. gertrudae</i>	<i>F. s. catus</i>	<i>R. exulans</i>	<i>R. rattus</i>
<i>T. gertrudae</i>	0.53			
<i>F. s. catus</i>	0.94	0.43		
<i>R. exulans</i>	0.81	0.95	0.82	
<i>R. rattus</i>	0.76	0.92	0.79	0.76

**Table 4.** Analysis of inter and intra species diet dissimilarities. Results of the Tukey HDS test for significant difference between species pairwise comparisons.

	Difference	Lower CI	Upper CI	$P_{adjusted}$
<i>T. gertrudae</i> – <i>F. s. catus</i>	0.08	0.04	0.12	< 0.001
<i>T. gertrudae</i> – <i>R. exulans</i>	-0.22	-0.27	-0.17	< 0.001
<i>T. gertrudae</i> – <i>R. rattus</i>	-0.16	-0.23	-0.09	< 0.001
<i>R. exulans</i> – <i>F. s. catus</i>	0.30	0.26	0.34	< 0.001
<i>R. rattus</i> – <i>F. s. catus</i>	0.24	0.18	0.30	< 0.001
<i>R. rattus</i> – <i>R. exulans</i>	-0.06	-0.12	0.01	0.13

**Table 5.** Prey availability and selectivity. Prey availability corresponds to the estimates of the number of prey individuals per Ha sampled over the six main habitat types of Niau Island. Jacobs electivity index ( $D$ ) is calculated for each predator.

	Prey Availability	<i>R. exulans</i>		<i>R. rattus</i>		<i>F. s. catus</i>		<i>T. gertrudae</i>	
		<i>MNI</i>	$D$	<i>MNI</i>	$D$	<i>MNI</i>	$D$	<i>MNI</i>	$D$
Scincidae	164	41	<b>0.81</b>	22	<b>0.67</b>	15	0.18	228	<b>0.96</b>
Coleoptera	219	43	<b>0.76</b>	24	<b>0.61</b>	39	<b>0.48</b>	224	<b>0.95</b>
Hymenoptera	483	164	<b>0.89</b>	130	<b>0.86</b>	18	<b>-0.26</b>	100	<b>0.72</b>
Orthoptera	339	9	-0.04	6	-0.21	13	-0.25	0	<b>-1.00</b>
Diptera	3552	117	0.09	220	<b>0.53</b>	5	<b>-0.96</b>	0	<b>-1.00</b>
Hemiptera	4896	3	<b>-0.97</b>	6	<b>-0.93</b>	0	<b>-1.00</b>	2	<b>-0.98</b>
Dictyoptera	113	68	<b>0.92</b>	51	<b>0.90</b>	139	<b>0.91</b>	87	<b>0.92</b>
Dermoptera	1	8	0.99	3	0.98	86	<b>1.00</b>	21	<b>1.00</b>
Lepidoptera	317	30	<b>0.55</b>	19	0.39	15	-0.15	0	<b>-1.00</b>
Odonata	104	0	<b>-1.00</b>	0	<b>-1.00</b>	3	-0.38	6	0.20
Scorpiones	1	2	0.97	1	0.95	6	0.98	1	0.93
Aranea	1948	6	<b>-0.82</b>	2	<b>-0.93</b>	0	<b>-1.00</b>	41	<b>-0.31</b>
Amphipoda	2725	3	<b>-0.94</b>	6	<b>-0.87</b>	0	<b>-1.00</b>	0	<b>-1.00</b>
Isopoda	3693	1	<b>-0.98</b>	4	<b>-0.94</b>	0	<b>-1.00</b>	0	<b>-1.00</b>
Myriapoda	4	31	<b>0.99</b>	6	0.96	9	0.95	0	<b>-1.00</b>
Rat	2	2	0.94	4	0.97	829	<b>1.00</b>	0	<b>-1.00</b>

## Diet overlap

Interspecific niche separation was highest between cats and the three other predators ( $MD = 0.92, 0.94$  and  $0.95$  for *R. rattus*, kingfisher and *R. exulans*, respectively), intermediate between *R. exulans* and both kingfisher and *R. rattus* ( $0.81$  and  $0.79$ , re-

spectively) and lowest between kingfisher and *R. rattus* (0.76) (Table 3, Figure 2[2]). These results are consistent with the calculation of diet overlap between predators. Diet overlap was almost complete between the two rat species ( $DO = 0.98$ ,  $CI = 0.96–0.99$ ), intermediate between kingfishers and rats (0.63  $CI = 0.56–0.70$  and 0.57  $CI = 0.48–0.69$  with *R. exulans* and *R. rattus*, respectively) and low between cats and the three other predators (0.21  $CI = 0.15–0.27$  with both Niau kingfisher and *R. rattus* and 0.17  $CI = 0.13–0.20$  with *R. exulans*). The prey taxa contributing most to diet overlaps between the Niau kingfisher and both rat species were Dictyoptera and Hymenoptera (*components A* > 0.86, *B* > 0.33), while *R. rattus* strongly shared two additional prey with this bird (Scincidae and Coleoptera; *components A* > 0.86, *B* > 0.33) (Table 1). Diptera, Myriapoda, Lepidoptera and Orthoptera were strongly shared by the two rat species (*components A* > 0.85), while *R. rattus* only significantly shared Gastropoda with cats (*components A* > 0.86).

## Discussion

This study is the first to jointly analyse the diet of an endemic island bird and three of the most harmful introduced predators. We sought to explore complex trophic interactions between native and introduced species on multi-invaded islands and to assess the impact of introduced predators on survival of the critically endangered Niau kingfisher.

### First reliable data on Niau kingfisher feeding ecology

Our study offers the first detailed diet analysis of the Niau kingfisher during its chick-rearing period, adding to the limited existing data. Our findings are crucial for the accurate conservation and management of this critically endangered bird.

First, the Niau kingfisher consumes a narrow range of prey taxa, but in regular abundances (i.e. low diet richness but relatively high diversity). Dissimilarity of diet samples is low, suggesting a relatively homogeneous diet. These results support a narrow diet breadth and specialised diet at a population level that make the kingfisher highly vulnerable to exploitative competition for its few main prey.

Second, Gekkonidae, Scincidae, terrestrial arthropods (principally Coleoptera, Dictyoptera and Araneae) and small Decapoda represent crucial resources for adult, nestling and fledgling kingfishers. In particular, Scincidae, Coleoptera, Hymenoptera and Dictyoptera are positively selected (i.e. consumed more than proportionate to their availability in the environment), suggesting that these scarce prey may be potentially at risk for exploitative competition with introduced predators.

Third, some of the Niau kingfisher's main prey are cryptogenic species, probably introduced from South-East Asia by Polynesians over the last centuries (Fisher 1997; Austin 1999; Ineich et al. 2007; Hamilton et al. 2010): *Emoia cyanura*, *E. impar*, *Lipinia noctua*, *Cryptoblepharus poecilopleurus* (Scincidae), *Lepidodactylus lugubris*,

*Gehyra oceanica*, *G. insulensis* (Gekkonidae) or more recently (late 1980s) for *Hemidactylus frenatus* (Case et al. 1994; Ineich et al. 2007). The latter species, generally considered as aggressive, has the potential to strongly compete and negatively impact local gecko populations (Case et al. 1994). The Niau kingfisher also widely consumes the cosmopolitan Dytiscidae *Pycnoscelus surinamensis*, *Blatella germanica* and *Periplaneta australasiae*, probably more recently introduced in the Tuamotu, at least partly from South Asia (Cochereau 1966; Parker et al. 1977). It is worth noting that the kingfisher likely shifted its nesting and foraging habits with the development and intensification of copra farming on the island, from patches previously dominated by the native palm tree (*Pritchardia mitiarioana*) to areas widely planted with the cryptogenic coconut tree on which this bird currently depends (Coulombe et al. 2011; Thibault and Cibois 2017). Hence, our study illustrates the positive function (e.g. alternative food or habitat resource) which exotic species can perform for island wildlife, particularly in man-transformed landscapes (e.g. Schlaepfer et al. 2011, 2012). Such complex interactions between native and introduced species, often scientifically neglected, need to be considered in restoration and conservation projects to prevent unexpected cascade effects from alien species removal or control.

### **Trophic interactions between the Niau kingfisher and the three introduced predators**

#### No support for high predation pressure on Niau kingfisher population

Although our study used two complementary approaches (morphological and PCR-based methods) to analyse a large number of cat and rat diet samples (578 cat scats and 295 rat digestive tracts), collected within kingfisher territories during the critical incubating and rearing periods, no bird remains were formally identified as Niau kingfisher. Our results suggest that, if predation by cats and rats does occur, it is much less frequent than suggested by Kesler et al. (2012).

To explain the decline of the Niau kingfisher, Kesler et al. (2012) hypothesised that the low survival of adult females (ca. 40%) might be due to nocturnal rat predation of nests during incubation. This hypothesis is not consistent, however, with the aggressive and territorial behaviour reported for Niau kingfishers, observed chasing rats from nesting trees (Gouni et al. 2006; pers. obs.). Since eggs are rarely left unattended (parents take turns brooding), unlike nestlings (parents often out foraging; G. Coulombe pers. comm.), the nestling stage could be the most vulnerable to rat predation. However, our results do not support this alternative hypothesis either. Kesler et al. (2012) also suggested that cat predation may be responsible for the high mortality of kingfisher juveniles (approximately 90% each year). Although cats are efficient bird predators (Doherty et al. 2016; McCreless et al. 2016) and the behaviour of juvenile kingfishers increases their predation risks (e.g. perching near the ground, calling loudly; Gouni et al. 2006; Kesler et al. 2012; pers. obs.), the absence of kingfisher remains in cat scats do not support this hypothesis either.

## Diet overlaps and potential exploitative competition between native and introduced predators

An extensive overlap in diet and food habits may indicate either a high potential for competition between species or a very abundant resource (Cupples et al. 2011). Evaluating whether the interacting predators select their shared prey more than proportionate to their availability in the habitat (positive selection) is therefore critical to untangle these two hypotheses.

On Niau Island, cats presented the narrowest diet breadth and the lowest variability in intraspecific diet composition, suggesting relatively homogeneous and specialised trophic behaviour (low prey richness with few abundantly preyed taxa) of individuals. Although cats and Niau kingfishers shared positively-selected prey (mainly Gekkonidae, but also Coleoptera, Dicyoptera and Dermaptera), their diets only marginally overlapped (DO = 0.21), making competition or competitive exclusion unlikely.

Conversely, both rat species presented generalist trophic behaviour, with the widest diet breadths and inter-individual variability in diet composition. Our study revealed a substantial niche overlap between the Niau kingfisher and both rat species (DO = 0.63 and DO = 0.57 for *R. exulans* and *R. rattus*, respectively). While Dictyoptera and Hymenoptera constitute the main diet overlap between kingfishers and both rat species, Scincidae and Coleoptera are also highly shared by *R. rattus* and kingfishers. Importantly, of the Niau kingfisher's prey, all but Dermaptera were significantly positively selected by both rat species (and more intensively by *R. exulans*), indicating possible exploitative competition with the kingfisher for these highly nutritive and relatively scarce prey (see Table 5). This exploitative competition may reduce the density and availability of Niau kingfisher critical prey ( Towns 2002; Rankin et al. 2018), thereby possibly affecting survival at different life-stages (adults, nestlings and fledglings) and/or breeding success.

## Conclusion and conservation perspectives

A better understanding of the complex and multiple trophic relationships between endangered natives (here, the Niau Kingfisher) and different invasive alien species should enhance decision-making on invasive species removal for conservation purposes. It should also help to anticipate potential deleterious cascading effects in trophic webs.

Although we do not question the important role that predation by introduced mammalian predators may have played in the past decline of the Niau kingfisher, our results fail to support the hypothesis of a current intense and continuous direct predation on this species. Conversely, our results reveal a substantial diet overlap between the Niau kingfisher and both rat species, suggesting an indirect impact by exploitative competition on key prey taxa (including cryptogenic and introduced species). Considering the critical size of the sole existing population of Niau kingfishers, it is important to avoid any additional mortality due to key prey rarefaction (or even direct preda-

tion). For these reasons, Niau Island was recently listed amongst islands worldwide where introduced mammal eradications are required to prevent imminent extinction of endemic vertebrates (Holmes et al. 2019).

Since rats represent the main prey of cats on Niau Island, cat eradication risks at least temporarily boosting rat populations, with the ensuing impacts on kingfishers from predation and competition (e.g. Courchamp et al. 2003; but see Bonnaud et al. 2010). Thus, the optimal and most time- and cost-efficient management action would be to simultaneously remove cats and both rat species to avoid a potential pernicious trophic cascade (e.g. Zavaleta et al. 2001). This would simultaneously decrease predation risk and increase key prey availability, likely to boost *T. gertrudae* population dynamics (Kesler et al. 2012). If such a triple eradication cannot be programmed due to logistics, sociological and/or financial issues, an alternative emergency interim strategy would be to locally control feral cats and rats (e.g. using live or kill traps, bait stations with toxins, feral cat shooting) within the Niau kingfisher's breeding and foraging areas, possibly combined with sterilisation of domestic cats and rat density control in the village, to slow down the recolonisation process of treated areas. In both cases, special care should be taken to (i) monitor the impact on the Niau ecosystem of increased introduced and cryptogenic prey densities following introduced predator management and (ii) reinforce biosecurity to avoid introduced mammal recolonisation of the island (e.g. from the port and airport). The public awareness campaigns conducted by local NGOs (e.g. SOP Manu) and collaborations with local farmers (nesting tree protection with rat-proof metal rings, promotion of mechanical clearing methods rather than fire) should be pursued, to enhance Niau kingfisher conservation.

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## **Supplementary material 1**

### **Estimates of prey relative availability and selectivity on Niau Island**

Authors: Diane Zarzoso-Lacoste, Elsa Bonnaud, Emmanuel Corse, Vincent Dubut, Olivier Lorvelec, Hélène De Meringo, Coralie Santelli, Jean-Yves Meunier, Thomas Ghestemme, Anne Gouni, Eric Vidal

Data type: species data

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Link: <https://doi.org/10.3897/neobiota.53.35086.suppl1>

## **Supplementary material 2**

### **Diet dataset obtained through morphological analyses**

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Data type: measurement

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Link: <https://doi.org/10.3897/neobiota.53.35086.suppl2>

## **Supplementary material 3**

### **Diet composition and breadth of the four studied predators**

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Data type: measurement

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**Supplementary material 4****Analysis of species richness and diversity**

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Data type: statistical data

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